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THE CLASSIFICATION OF LICHENS

By W. WATSON

PART I

GENERAL DISCUSSION

WHEN lichens were recognised as organisms distinct from algae or fungi they were arranged according to their external features into fruticose, foliose and crustaceous groups. Some lichens, in which the thallus was gelatinous when moist, were placed by some lichenologists⁽³⁰⁾ in a fourth group: the gelatinous lichens. This method was a useful one in the times prior to the elaboration of the compound microscope and enabled a large number of our common lichens to be discriminated. In the determination of lichens in the field it is of great service to-day, especially in regard to those lichens in which spore-producing bodies are seldom or never found, but the increased efficiency of the microscope and its manipulation in investigating the characters of the plant has caused the method to be almost entirely superseded by a more natural one in which all the characters are taken into account. Harmand's *Lichens de France* (1905-13), which unfortunately was never completed, followed this old method to a great extent, but is probably the last serious attempt to modify a moribund taxonomic system so as to make it accord with our increasing knowledge of the structure and life history of lichens. There was a time in the history of phanerogamic taxonomy when the external and vegetative characters were considered of primary importance, but complete emancipation from these views has now been largely achieved; internal and reproductive characters have been shown to be the most constant ones and their use is now held to be a leading principle in a natural classification. Complete emancipation from such ideas in lichen taxonomy is far from achieved, though it has been partially accomplished.

A lichen is generally considered to be a plant in which algal cells and fungal filaments are so intimately associated that an autonomous

organism is formed and this is able to live under conditions which are unfavourable or even fatal to one or both partners. During vegetative growth the partners are of similar importance to the organism, the algal cells obtain the carbonaceous material whilst the fungal filaments obtain water and the inorganic salts dissolved in it. In vegetative reproduction the partners are again of similar importance, algal cells being wrapped round by fungal filaments so as to produce a soredium. When reproduction is effected by means of spores the process is controlled by the fungal partner, the subsidiary rôle of the algal partner being to help in the supply of food and the environment necessary for the formation of the spore-producing body. The spore essentially belongs to the fungal partner but is usually so modified that it can only develop into an autonomous plant when the appropriate alga is supplied and then it forms a plant similar to the parent lichen.

This view of the lichen follows, in a general way, the dual hypothesis propounded by Schwendener (31) and modified by later lichenologists. Other views, such as those of Church (4), Elenkin (9) and Danilov (7), Moreau (24), Fink (10) and McWhorter (23), have not received any general support from lichenologists (28, 32a, 33).

In accordance with these views of lichen organisation a natural classification must take account of both symbionts but, as the sexual and asexual methods of reproduction are chiefly concerned with the formation of the fungal partner, the characters of the latter should be considered of greater importance than the nature of the algal one. The method of formation of the thecium, its shape and the production and septation of the spore are now considered of greater importance than the algal symbiont or the form which the vegetative thallus assumes. The probable phylogenetic history must also be co-ordinated with any attempt at classification. As I have written previously (1912), "a system of classification should take into account not only the present structure of the plants involved, but also their evolutionary history, interpreting the former in terms of the latter. This is a complicated business in a composite group such as the lichens, for even if the ancestors of the (fungal) symbionts have not disappeared, the modifications induced by their mutualism may have been so great that it is now almost impossible to trace them" (35a).

Acharius (1), as early as 1803, made use of the apothecium in his classification, whilst Nylander (27) in 1854 and 1858 used both thalline and thecial characters in his complicated scheme. He almost entirely neglected the spore characters and, despite the great service rendered

by him to lichenology, his scheme has few sympathisers to-day. His system is of interest to British lichenologists as it was followed by Leighton^(17b) and Crombie⁽⁸⁾ in their works on British lichens. It is only in recent years that the shackles of this slavish attachment to Nylanderian conceptions have been broken by British lichenologists^(32a). The importance he attached to the spermogones and spermatia is not warranted by the present-day knowledge of these structures, and their occurrence is too uncertain and limited for their characters to be considered important for taxonomic purposes. If one considers them as male gametangia and gametes, which are sometimes functional though usually functionless, they have a certain value, especially in doubtful cases of affinity, but they are too frequently absent in many lichens to be of general taxonomic value. On the theory that they are pycnidia and pycnidiospores their value is lessened when one considers the variability of such accessory asexual structures in the fungi. Nylander's work on the spermogonia was supplemented in this country by that of Lindsay⁽²⁰⁾, but, as Lorrain Smith remarks^(32c) p. 205, "in many instances he must have been dealing with species of the 'Fungi imperfecti' that were growing in association with the scattered granules of crustaceous lichens." This proneness to inaccuracy when dealing with spermogonia seriously discounts their use in a general scheme of classification.

Leighton, in 1851, was one of the first lichenologists to pay particular attention to the spore, as in his account of the British angiocarpous lichens^(17a). A little later Massalongo and Koerber⁽¹⁶⁾, among continental lichenologists, evoked a large amount of hostile criticism by using the spore as a generic distinction. They were followed by Mudd, who in his *Manual of British Lichens*⁽²⁵⁾, anticipated many of the generic names which are in general use to-day.

The classification proposed by Müller-Argau⁽²⁶⁾ in 1862, and the important contributions of Reinke⁽³⁰⁾¹ in 1894-6 made some advances towards the development of a natural system of classification of lichens, but the most successful attempts have been made by Zahlbruckner whose system has, to a large extent, been followed by British lichenologists. It is based on all the characters of the dual plant though the thecia and the spores contained in them have a dominating influence. Instead of the old divisions founded upon the

¹ Reinke's treatment of the subject was far superior to that of Müller-Argau. The progress which has been made towards a natural scheme of classification is largely due to his influence.

fruticose or foliose or crustaceous character of the thallus a sounder classification has been gradually evolved and Zahlbruckner has embodied most of the advances made in his taxonomic scheme.

The few lichens, in which the spores are borne on basidia, form the sub-class Basidiolichens (Hymenolichens), whilst the great majority of lichens, in which the spores are produced in asci, constitute the sub-class Ascolichens. The latter are divided into two groups: (1) Gymnocarpeae, with a thecium (apothecium) exposed at maturity, and (2) Pyrenocarpeae, with a thecium (perithecium) in which the hymenium is not exposed or communicates with the exterior by a small apical pore only. The first group is a large one and, as it includes lichens with three types of apothecia, three groups, Coniocarpineae, Graphidineae and Cyclocarpineae, are recognised. In the first group the apothecia are usually stalked and, at maturity, the spores leave the asci and form a sporal mass (mazedium) on the surface. In the second the apothecia are elongated or with a disc more or less slit-like. In Cyclocarpineae the apothecium has an open orbicular \pm disc at maturity. These groups may be considered as analogous to orders in other groups of Thallophyta but not as exactly corresponding. The arrangement of lichens in them does not necessarily imply that all the members of a particular order have been derived from a common ancestor. The usual inferences from the use of the term "order" cannot be made owing to the probability that there have been many starting-points of consortia. Fungi enter into symbiotic union with many plants of different phyla, not only with algae, but also with liverworts, ferns and flowering plants, and, in some cases, the union is an obligate one⁽²⁹⁾. On Mendelian principles it is difficult to group lichens at all, the consortium and its origin being so difficult to interpret. In the familiar cases of orchids and mycorrhizas the origin of the consortium was probably due to a mild parasitism of the fungus on the higher plant⁽²⁹⁾, but in the lichen the origin is to be sought for in the close association of two independent plants of similar status. How this has resulted in the obligate symbiosis of a lichen plant and how the factors for producing the consortium have been impressed in the fungal spore are questions of great interest to students of heredity but, in the present state of our knowledge, need not necessarily concern the systematist. The fact that so many plants are able to enter into a temporary or permanent symbiosis with fungi does, however, show that a polyphyletic theory of the origin of lichens is quite sound. It may, perhaps, be possible, when the conditions governing germina-

tion are better understood and sufficient skill in manipulation of cultures obtained, to produce an entirely new lichen by the synthesis of an alga and a fungus. So far as is known a completely new synthesis has not been accomplished between a fungus and another plant in the numerous experiments on mycorrhizas. Bachmann has recently given an account of the establishment of mutualistic relations between a foreign fungus and the algal cells in the thallus (podetial) of a *Cladonia*. The algal cells multiplied to a great extent, the fungal hyphae were stimulated and a gall-like body was formed (2).

The probability that there have been many independent origins of the consortia, together with the relationship of the spore to the fungal constituent, has led some botanists to discard lichens as a class, and some attempts have been made to relegate them to appropriate positions amongst the fungi. Lichenologists are not prepared to admit this general subordination. The mutualistic relations between the fungus and the alga result in what is usually a complex and entirely different type of plant, whose life depends as much on the alga as the fungus, except during the formation of the thecium. In this new plant, evolutionary development has occurred. Many lichens are also partially or altogether dependent for their continuance on their vegetative methods of reproduction, and in these the two symbionts are of similar importance.

An order of lichens then, is merely a group in which families sufficiently similar can be arranged together. Lichenologists accept the thecia as the taxonomic bases of these "orders," which are mostly considered as polyphyletic. When the fungal constituents of two lichens (containing the same alga) are distinctly different, one can generally assume that two independent associations of alga and fungus have originated the consortia. It must not be overlooked, however, that variation and evolution may go on in the fungal partner after symbiotic union has been established. The likeness between the fungal symbiont of a lichen and a free-living fungus of the present day does not necessarily mean that the latter is the representative of the former, since the resemblance may be due to convergence.

When the algal constituents of two lichens (containing the same fungus) are different, it is often assumed that the consortia have had quite independent origins from the association of free-living algae and fungi. This may not be exactly correct, since there is a possibility that the germinating spore of the first lichen may have been able to enter into partnership with another alga so as to produce the second lichen.

The Cyclocarpineae are a large group which has been rendered less cumbersome by some lichenologists who have followed Reinke (30) in taking away from it those lichens which have Cyanophyceous algae as their algal symbiont, and putting these under a separate group Cyanophila. This group has a thallus which is usually more or less gelatinous owing to the nature of the algal symbiont. It includes a few plants with green algal cells, but otherwise so agreeing in general characters with species containing the usual blue-green algae as to suggest a similar phylogenetic origin, at any rate in regard to the species compared. By the adoption of this method the algal symbiont seems to have a taxonomic importance similar to that of the fungal one and a modification will be proposed, later on, which will divide Cyclocarpineae into five secondary groups. It may, however, be mentioned here that Zahlbruckner seems to recognise the group Cyanophila, in a partial way, by the manner in which he arranges the families belonging to the Cyclocarpineae. The families with blue-green algae are sandwiched between two lots of families with algal cells belonging to Chlorophyceae. Of these three lots the first one consists largely of families with primitive or homoiomerous thallus, then follow the families with blue-green algae, whilst the last lot consists of families in which the thallus is usually heteromerous. It may also be noted that the Cyanophyceous lichen families are arranged so that those with heteromerous thallus follow those with homoiomerous.

The chlorophyceous lichens included in the Cladoniaceae have two kinds of thallus as, in addition to the ordinary one, they have a more or less erect podetium with a structure which is usually radiate. The podetium, in most cases, is usually taken to represent a development of an apothecial stalk into which algae have extended. Wainio believes that the members of Cladoniaceae had a monophyletic origin and Lorrain Smith agrees (32c) p. 293 that the "family is monophyletic in origin, though many subordinate phyla appear later¹." Though it is not difficult to derive them from some form of *Lecidea* or *Biatora* (32c) p. 293, it is probable that they form a group quite distinct from any other. The amount of variation they exhibit indicates the antiquity of the group, and even if *Lecidea* and *Cladonia* had a common ancestor, the evolution of their derived members has proceeded along divergent lines. Cladoniaceae has long been recognised as a group distinct from others: so long ago as 1780

¹ It is probable that these subordinate phyla are parallel developments and that "Cladoniaceae" has had three originating points, see p. 31.

Weber gave *Cladonia* as one of the 18 genera amongst which he distributed lichens, whilst Müller-Argau in 1862 recognised the *Cladonia* group (Capitularieae) as one of the three groups in his Eulichens.

On account of these considerations and others which will be mentioned later, it is proposed to divide Ascolichens as follows. The Pyrenocarpeae, Coniocarpineae and Graphidineae constitute the orders Pyrenocarpaceae, Coniocarpales and Graphidales respectively. The group Cyclocarpineae is such a large and varied one that it is more convenient to divide it into smaller groups, which may be considered as follows:

(1) Ectolechiales. Apothecia unstalked and of simple structure; thallus primitive and usually homoiomerous; algal cells belonging to Chlorophyceae.

(2) Collemales. Apothecia unstalked; thallus homoiomerous, usually more or less gelatinous; algal cells belonging to Cyanophyceae.

(3) Peltigerales. Apothecia unstalked; thallus heteromerous; algal cells normally belonging to Cyanophyceae.

(4) Parmeliales. Apothecia unstalked (or almost so); thallus heteromerous; algal cells belonging to Chlorophyceae.

(5) Cladoniales. Thallus heteromerous and of two kinds, the ordinary or primary and the podetial or secondary; algal cells belonging to Chlorophyceae. The podetial thallus is usually more or less erect, often has a radiate structure and, on it, the apothecia are borne.

Reinke's Cyanophila⁽³⁰⁾ includes Collemales and Peltigerales, Ectolechiales consist of those lichens arranged by Zahlbruckner before the cyanophyceous ones whilst Parmeliales and Cladoniales follow them.

It must again be noted that the division of Cyclocarpineae into five groups does not necessarily imply a separate monophyletic origin for each group, though such a desirable objective is brought appreciably nearer. The five groups must be regarded as convenient for placing together families possessing similar characters.

Ectolechiales are certainly a primitive group in regard to the thallus but the possession of a homoiomerous thallus cannot be taken as a rigid character for purposes of classification. Some Ectolechiale families may possess members in which the algal cells have become arranged in a definite layer whilst some members of the Parmeliale group may remain in a primitive thalline condition. From the Ectolechiale families the Parmeliale families were probably

derived, and good reasons may be advanced for including many members of the Ectolechiales as primitive members of Parmeliale families.

Collemales may be a primitive group, or they may have been derived from the Ectolechiales by the substitution of a cyanophyceous alga for a chlorophyceous one. That they have not advanced to stages higher than a foliose one may be due to a smaller capacity for advancement when the algal component belongs to the simpler alga or, on the other hand, may be due to their later development. If the former view is taken the taxonomic value of their homioimerous thallus is considerably increased, especially if the Peltigerales are regarded as derivatives from the Parmeliales. Then the Collemales may be considered to be a primitive group in which the thallus containing blue-green algae does not usually advance beyond the homioimerous condition.

Peltigerales may be considered as advances from the Collemales or as derivatives from Parmeliales. The similarity of certain genera (or subgenera, *section* of many lichenologists) containing different algal cells lends some support to the latter view. For example, in *Lobarina scrobiculata* the algal cells are blue-green, whilst in the similar plant *Lobaria* (*Ricasolia*) *laetevirens* they are bright green. Lichenologists are in general agreement in regard to the close affinity between these two plants. If one assumes the correctness of this view with its phylogenetic implications, *Lobaria* (*Ricasolia*) *laetevirens* may be considered as near to the ancestral form whilst *Lobarina scrobiculata* has been derived from it, owing to the germinating ascospore of the ancestral form entering into mutualistic relations with a blue-green alga. On the other hand, the *Lobaria* may have originated from the *Lobarina*, by the substitution of green for blue-green algal cells¹. In any case the group, as it exists to-day, is a convenient one in which to place the families Peltigeraceae, Stictaceae and Pannariaceae.

Parmeliales contain the lichens with the greatest capacity for thalline advancement. The names Lecideales and Lecanorales have been formerly employed, in part, for them, but not in the sense in which Parmeliales is here used. These names (Lecideales and Lecanorales) implied a great taxonomic value to the absence or presence of a thalline margin to the apothecium. As this character is not used in the separation of Parmeliales from the other Cyclocarpineae, neither name is applicable to this group.

These five groups of Cyclocarpineae may be considered as sub-

¹ Further examples of possible substitution are given on pp. 16-17.

orders or as orders. The chief objection to the use of the latter term (in addition to the phylogenetic implication previously mentioned) is that thalline characters, as well as thecial ones, are used as taxonomic bases, so that these "orders" are not commensurate with Pyrenocarpales, Coniocarpales and Graphidales in which thecial characters form the main bases of classification. As a matter of convenience their consideration as orders is justifiable.

In the further separation into families the taxonomic importance of the apothecia and the spores are again emphasised by Zahlbruckner. As regards the importance attached to spore characters the most striking example is afforded by the treatment of the present-day genera of *Teloschistes*, *Xanthoria*, *Caloplaca* (*Placodium* and *Callophisma*), *Blastenia*, *Anaptychia*, *Physcia*, *Rinodina* and *Buellia*. In many lichenological works the thalline characters of these plants largely determine their positions. The fruticose *Teloschistes*, the more or less fruticose *Anaptychia* and the foliose *Xanthoria* were included together under the foliose *Physcia*. Because their thalli were \pm crustaceous *Caloplaca*, *Blastenia* and *Rinodina* were put under *Lecanora*, whilst *Buellia* was placed with *Lecidea*. In these lichens Zahlbruckner seems to consider the colour and septation of the spore as of great importance. Those having colourless and polarilocular spores are placed in the neighbouring families of Teloschistaceae (*Teloschistaceae*) and Caloplacaceae, the former containing the fruticose *Teloschistes* and *Lethariopsis*, and the foliose *Xanthoria*, whilst the latter contains the \pm crustaceous *Caloplaca* and *Blastenia*¹. Those having one-septate and brown spores constitute the allied families Physciaceae and Buelliaceae, the former containing the fruticose or foliose *Anaptychia*, *Physcia* and *Pyxine*, whilst the latter contains the crustaceous *Rinodina* and *Buellia*.

This consideration of the apothecium and its contained spores as of great importance seems to be in accordance with the habits of crustaceous lichens. In many of these the thallus is either evanescent or poorly developed, and in many others in which a thallus is normally present, there are varieties or forms in which the thalli are so poorly developed that they render no aid to their identification. This is especially the case with lichens occurring in the arctic or alpine regions, the thallus often being so scanty that the characters of the apothecium form the only means of determination. "Some lichens

¹ The inclusion of the simple-spored *Protoblastenia* and the 3-many-septate-spored *Bombyliospora* in the family Caloplacaceae, shows that he does not consider the septation of the spore as of paramount importance.

...living under arctic conditions of life...are reduced to hardly anything, some apothecia and scattered thin areolae(21). There are also innumerable cases of lichens, especially crustaceous ones, in which the thalline characters fluctuate to a considerable extent, the only constant characters being those of the apothecium and spore. Lichenologists therefore rely largely on the size, colour, number and septation of the spores in their determinations and it seems quite justifiable to attach importance to these characters for taxonomic purposes. This reliance for determination purposes on spore characters does not imply that they should override all others when taxonomic questions are considered, but it certainly suggests that much consideration should be given to them. This was recognised by Mudd, so long ago as 1862, when he wrote "of all organs furnished by a given group of plants, none offer so many real, constant, and physiological characters as the spores of the Lichens, for the formation of a simple and natural classification" (25).

Just as changes have occurred in other characters, the colour and septation of the spore may have changed during the phylogenetic development of a particular group, so that the sporal characters must be considered in conjunction with others. The view that septation changes in the spore have been frequent after the consortium has originated, is not favoured when we consider that the majority of the highly developed lichens have simple spores. Although the colour and septation of the spore are two of the most constant characters in lichens, they are subject to variation in some genera and even in some species. In *Rhizocarpon* the spore is colourless in some species and dark in others. In *Buellia* a dark-coloured spore is a generic character but there are some species (e.g. *B. colludens*) in which the spore is colourless up to, or almost up to, maturity. Inconstancy is also shown in the septation of the spore. *Rinodina* has dark 1-septate spores but *R. conradi* appears to be 3-septate owing to the partial development of two extra septa. A similar thing occurs in *Callopisma tetrastichum*, the spores of which, apart from their apparent 3-septate character, are very similar to the colourless polarilocular spores of the allied *C. ochraceum*. In *Rinodina diplinthia*, which is otherwise very similar to *R. conradi*, the spores are often described as becoming more or less muriform. *Biatorina* (with 1-septate colourless spores) is not always easy to distinguish from *Bilimbia* (*Weitenwebera*), in which the spores are also colourless but 3- or more-septate. An extra septum may be developed in the spores of some *Biatorinas* whilst some species of

Bilimbia do not show more than one septum except when full development has occurred. Some species of *Biatorina* also appear to belong to *Lecidea* (with unseptate spores) as a septum is not formed till the spores are completely mature. The appearance of longitudinal divisions in transversely-septate spores so as to give rise to muriform spores is inconstant in some cases (e.g. species of *Rhizocarpon*), many apothecia having spores with transverse septa only; full development so as to give rise to muriform spores only occur in some of the apothecia. On the other hand, an extra oblique or longitudinal septum occasionally occurs in species (e.g. *Thelidium cataractarum*) which are considered to have spores which are merely transversely 3-septate. In the same plant some perithecia may contain spores which are merely 1-septate or even unseptate. Many of these irregularities can be shown to be more apparent than real. In *Rinodina conradi* and *Callophisma tetrastichum* the 3-septate appearance of the spore may be considered as due to the constriction of the lumina, whilst some other irregularities are due to incomplete development. A sufficient number of real irregularities occur to show that one or more extra divisions may be formed. This is especially the case with spores having three or more septa, one or more extra longitudinal divisions frequently occurring. The easy nature of the passage from spores with many transverse septa to spores which are more or less muriform, suggests that it is unnecessary to attach much taxonomic value to such additional septation. It may be sufficient to put the species possessing longitudinal as well as transverse division into a different genus from those with transverse septation only, but it seems of doubtful value for placing them in different families.

It should also be noted that Zahlbruckner's classification sometimes cuts across the old line of demarcation between lecanorine and lecideine apothecia, as in Buelliaceae he includes the lecanorine *Rinodina* and the lecideine *Buellia*. In Caloplacaceae the species having a thalline margin to their apothecium are placed in *Caloplaca*, whilst those without are put in *Blastenia*. The presence or absence of a thalline margin to the apothecium does not deserve to have such a weighty significance attached to it as is done by some lichenologists, who, on this account, place in a particular family genera with various sporal characters. Zahlbruckner himself, in his families Lecanoraceae and Lecideaceae, seems to attach great importance to the presence or absence of a thalline margin. The apothecium has a thalline margin in his family Lecanoraceae which includes the simple-spored genera of *Lecanora*, *Harpidium* and *Ochrolechia* with the

septate-spored genera of *Lecania*, *Icmadophila*, *Haematomma*, *Phlyctis*, etc. His family Lecideaceae has the apothecium without a thalline margin and contains the simple-spored *Lecidea*, the uni-septate-spored *Catillaria* (*Biatorina*), the polyseptate-spored *Bacidia* (including *Bilimbia*) and the muriseptate-spored *Rhizocarpon*. In the same family Lorrain Smith^(32a) also includes *Buellia* with brown, 1-septate spores. Zahlbruckner recognises that the latter genus is better placed with *Rinodina* which merely differs in the possession of a thalline margin by the apothecium. He also follows in the wake of others in attaching little significance to the thalline margin in the families Caloplacaceae or Physciaceae, as he includes in them lichens with (e.g. *Physcia*), or without (e.g. *Pyxine*), a thalline margin to the apothecium. A similar course in regard to the presence or absence of a thalline margin is adopted by Lorrain Smith^(32b) for *Caloplaca* and *Blastenia*, all the species, whether they possess a thalline margin or not, being put under *Placodium*. Lichenologists often meet with great difficulty in deciding whether a thalline margin is present or absent, and have gradually realised that algal cells are present, around or under the apothecium, in many lichens which otherwise appear to have a lecideoid apothecium. In consequence of this difficulty there are diverse views in regard to the genus in which some species should be placed, e.g. *Lecania* or *Biatorina cyrtella*, *Rinodina* or *Buellia discolor*, *Lecanora* or *Lecidea coarctata*. A further argument against attaching too much importance to the thalline margin is that some species, which normally have a thalline margin to the apothecia, have varieties or states in which a thalline margin is absent, or, if present at first, quickly becomes obliterated, e.g. *Lecania erysibe*, *Lecanora sarcopisioides*, *L. sulphurea*, *L. polytropa*. The number of such cases is so great that one cannot attach such great importance to the method of emergence of the apothecial body, or to the occurrence of algae in its margin, as the old taxonomic systems demand. The difference is usually significant enough to warrant a generic separation as between *Lecanora* and *Lecidea*, *Lecania* and *Biatorina*, or *Rinodina* and *Buellia*, but not sufficient by itself to form the basis on which a family is founded. As stated previously in 1922^(35a), "too much importance may be assigned to the systematic value of the distinction between lecideine and lecanorine apothecia...and an extension of the principle which includes *Blastenia* with *Placodium*, and *Buellia* with *Rinodina*, seems justifiable."

In regard to the crustaceous members of Lorrain Smith's

Teloschistaceae (put under Caloplacaceae by Zahlbruckner), attempts to apportion them to genera with or without a thalline margin lead to much confusion. When both the thallus and apothecium are yellow the concolorous margin may belong to either, and there is so much uncertainty in deciding as to the presence or absence of algal cells, around or immediately under the apothecium, that it seems advisable to place all those which possess yellowish apothecia under one genus. This course was practically adopted by Lorrain Smith^(32b) when she used *Placodium*, though this was used in a wider sense still, including the radiately-lobed species as well as the truly crustaceous ones¹. Malme adopts a similar course but uses *Callophisma* instead of *Placodium* as the generic name⁽²²⁾. For many years *Callophisma* has been used by me for all the truly crustaceous species (as in ^(35 e)). When *Blastenia* is used as the generic name for those without thalline margins there is much divergence in the nomenclature, e.g. both *citrinum* and *phloginum* are put in the *Callophisma* section (or its equivalent) by Lorrain Smith and Zahlbruckner, whilst Lesdain puts them under *Blastenia*⁽¹⁸⁾. The difficulty of deciding whether the margin is a thalline or proper one when these are concolorous, is often so great that it counterbalances the convenience of reducing the size of the genus *Callophisma*.

Zahlbruckner further emphasises the taxonomic importance of the sporal reproductive bodies in another way. His family Acarosporaceae is founded chiefly on the presence of many spores in the ascus. The spores, with the possible exception of *Maronea*, are simple. The apothecium of *Acarospora* may be regarded as possessing a thalline margin whilst in *Biatorella* a thalline margin is never present. *Glypholechia* is more or less foliose whilst the other members are crustaceous or, at most, squamulose. Some lichens with polyspored asci are not included under Acarosporaceae because their structure in other respects suggests a different phylogenetic relationship. This applies to the foliose *Anzia* which was included by Reinke in this family.

Zahlbruckner therefore takes account of all the characters and considers the probable phylogeny in his attempts to found a natural classification, but is not altogether consistent in his methods. There seems to be no real reason why the method of treatment adopted for Teloschistaceae, Caloplacaceae, Physciaceae and Buelliaceae should not be extended in regard to other lichens. For family characters

¹ These are placed under the sub-genera *Callophisma*, with a thalline margin, and *Blastenia*, without a thalline margin.

the structure of the spore is considered to be important, the presence or absence of a thalline margin to the apothecium is disregarded and the thalline characters are treated as of secondary importance. The phylogeny of lichens is rather obscure but the phylogenetic relationship of most of the genera included in these four families seems fairly clear, whilst in his families Lecanoraceae and Lecideaceae some genera (see pp. 11-12), which seem to have little phylogenetic connection, are included. If a family containing both septate and unseptate spores has any phylogenetic significance, we must consider that its members have had additional septa developed in the spores during the course of descent from a common ancestor possessing a lecideine or lecanorine (as the case may be) apothecium and simple spores. Ontogeny indicates that this was how septation originated, but does not necessarily suggest that the process occurred after the fungus took on a partnership with the alga. In fact, the widespread occurrence of septation in pure fungi, and the presence of simple spores in many highly developed lichens, indicates otherwise, unless one assumes that the septate-spored fungi have originated since the establishment of most lichen consortia. In the suggested re-arrangement the fungal partner is generally regarded as having had a simple or septate (as the case may be) spore, the lecideine apothecium is considered as primitive, and the usual course of evolution was in the extension of the algae into the margin, or in the formation of an additional margin in which algae were present. As Lorrain Smith says (⁽³²⁾ p. 298), "the marginate apothecium has appeared once and again. . . . *Lecanora* must have originated when the crustaceous lecideine thallus was already well established. Its affinity is with *Lecidea* and not with any fungus: where the thallus is evanescent or scanty, its lack is due to retrogressive rather than to primitive characters."

The following table arranges some of the common genera according to their spore-characters and indicates that there are groups which are more or less parallel to the four families mentioned above.

The table shows that in this particular order of lichens (Parmeliales) those with 1-septate and colourless spores form a series almost parallel to those formed by Teloschistaceae and Caloplacaceae or by Physciaceae and Buelliaceae. This series includes the fruticose *Ramalina*, the squamose *Solenopsora* (*Placolecania* or *Diphratara*), the squamulose *Thalloidima* and the crustaceous *Lecania* and *Biatorina*, *Lecania* having a thalline margin to the apothecium whilst *Biatorina* has not. There does not seem to be any reason (if

Tabular arrangement of some genera of lichens (Parmeliales) so as to show how different types of spore are found in different types of thallus.

	Thallus is squamose or squamulose		Thallus is crustaceous	
	Apothecia with thalline margin in	Apothecia without thalline margin in	Apothecia with thalline margin in	Apothecia without thalline margin in
Spores are usually 8 in the ascus				
Colourless and polarilocular in	Telochistites Lethariopsis	Xanthoria	Placodium (Gasparrinia)	Calopisma Blastenia
	Teloschistaceae		Caloplaceae	
Dark and 1-septate in	Anaptychia	Physcia Pyxine (with lecidoid apothecia)	Placothallia (=Dimelaena)	Rinodina
	Physciaceae		Buelliaceae	
Colourless and 1-septate in	Ramalina			Buellia
			Solenopora (=Placotecania) Callopiis Trichoplasia (2-septate)	Thalloidima
Colourless and simple in	Usnea Letharia Evernia and Everniopsis Cetraria Alectoria Dactylina Dufourea Oropogon (muriform spores)	Parmelia Parmeliopsis Platyria Nephromiopsis Heterodea	Squamaria	Psora Phyllopsora
			Physcidia (many-septate spores)	Toninia (3- or more-septate spores)
Colourless or dark, 3- or more-septate to muriform in		Psorella (spore 3-15-septate and apothecia lecidoid) Megalospora (0-3-septate and acicular spores)		Haematomma (3-many-septate) Myxodictyon (muriform)
				Bilimbia Bacidia Dombyliopsis Rhizocarpon (muriform spores)
				Lecanora Ochrolechia Aspicilia Jonaspis Harpidium (thallus homoi- omorous)
				Lecidea Orpiniospora (spores brown) Mycoblastus
				Biatolina Catillaria Megalospora Microphiale

the principle of including *Callopisma* and *Blastienia* in the family Caloplacaceae, or *Rinodina* and *Buellia* in the family Buelliaceae is sound, as it seems to be) for placing *Lecania* and *Biatorina* in different families. Their relationship is so close that some species (e.g. *Biatorina cyrtella*) are placed in *Biatorina* by some authors and in *Lecania* by others. These two genera and their near allies may then be grouped together in a family called Lecaniaceae. The squamose *Solenopsora* may be included with them but the fruticose *Ramalina* seems sufficiently distinct to form a family of its own. The two lichens formerly known as *Biatorina lutea* and *B. diluta* (32a) seem similar to other *Biatorinas* in their reproductive characters, but differ in having *Trentepohlia* as their algal symbiont. On this account they have been placed by Zahlbruckner under the genus *Microphiale* and included in the family Gyalectaceae. When the reproductive characters are considered as of primary importance *Microphiale* must be placed in the same family as *Biatorina*. This arrangement implies that *Microphiale* has been derived from *Biatorina* by the substitution of a red algal symbiont for the more usual green one. That this is quite a probable method of evolution is shown by the consideration of some other cases where one algal symbiont is more or less replaced by another. The standard cases of this partial replacement (or addition) are those which occur in *Lecanora granatina* (11b) and *Solorina* (with green algal cells). In some species of *Solorina* groups of blue-green algae are scattered in the lower part of the thallus, or in special squamules, whilst in *S. crocea* they form a fairly continuous stratum below the layer of the ordinary green algal cells. According to Forsell (11) a similar thing occurs in *Psoroma hypnorum*. The squamules formed are very similar to those of *Pannaria pezizoides* (with blue-green algal cells) and this suggests that the latter may be a substitution product of the *Psoroma*. The taxonomic treatment of many other lichens shows that the inclusion of genera having two different algal symbionts in the same family breaks no canon of lichenological classification. *Sticta* and *Stictina* are often included in the same genus (*Sticta*) though they differ in the algal constituent. *Lobaria*, in its broad sense, includes species with green algae (in *Lobaria* and *Ricasolia*) or with blue-green (in *Lobarina*). *Arthonia*, of many authors, includes species which possess green algal cells instead of the more usual *Trentepohlia*. In the family Byssolomaceae (sec. Zahlbruckner) *Byssoloma* and *Amphischizonia* have green algal cells, whilst *Asteristion* contains *Trentepohlia*. The family Thelotremaceae (sec. A. L. Smith (32b)) also includes genera with these two kinds of

algal cells. That substitution of the algal cells is possible is also implied by the casual invasion of foreign algae, as in *Lecidea uliginosa*, though it is doubtful whether any benefit results from their inclusion in any examples which have been investigated. *L. uliginosa* is often very abundant on peaty ground and, occasionally, blue-green algae occur in the thallus along with the ordinary green ones. A similar phenomenon has been noted in other lichens.

The probability that these foreign algae give no benefit to the lichen, or, at any rate, do not enter into relationship with the hyphae, does not invalidate the possibility of substitution being an evolutionary method. At an early period in the history of the lichen, the relationship between the alga and fungus may have been less definitely fixed, and the invading alga may have become the paramount algal partner, or may even have entirely replaced the original one. In the simple-spored group, *Jonaspis* differs from *Aspicilia* in having reddish algal cells instead of green. It has been placed, on this account, in the family Gyalectaceae by Zahlbruckner but, apart from its algal symbiont, has no relationship with *Gyalecta*, and from the considerations advanced above should rather be regarded as an aberrant *Aspicilia*. The table also shows that there are two other series, which are more or less parallel to those accepted by Zahlbruckner. In one of these series the spores are simple and in the other they are 3- or more-septate.

The simple-spored group includes such a large number of genera that it seems advisable for several families to be formed from it. *Usnea*, on account of its solid axis, almost merits a family to itself, but *Letharia* is sometimes more or less intermediate in this respect so as to link it to *Alectoria* with a "webbed" axis. When *Ramalina* and *Oropogon* are excluded, the family Usneaceae (of Zahlbruckner) can be accepted as conforming to the sporal requirements though *Thamnolia* (*Cerania*), *Siphula* and *Endocena* remain doubtful members, since their apothecia are unknown. The family Parmeliaceae (sec. Zahlbruckner) has two genera (*Megalospora* and *Physcidia*) attributed to it, which on account of spore-characters are better omitted. *Candelaria*, despite the many-spored ascus and the absence of parietin, appears to be phylogenetically related to *Caloplaca* (*Placodium*) and should be transferred to Caloplacaceae. Its spores, though simple, often simulate the polarilocular spores of other members of the family. The families Lecanoraceae and Lecideaceae require emendation, the septate-spored genera being omitted. *Candelariella*, for reasons similar to those advanced above in regard

to the inclusion of *Candelaria*, should be put under Caloplacaceae (see p. 21).

The following represents the proposed arrangement when the reproductive characters have sufficient importance assigned to them. Where the relations between the genera are better shown by means of a key, one is usually given.

Class LICHENS

Sub-class HYMENOLICHENS (BASIDIOLICHENS)

Order I. CORALES. *Cora*, *Dictyonema*, *Corella*

Sub-class ASCOLICHENS

- | | |
|---------------------------|--------------------|
| Orders II. PYRENOCARPALES | III. CONIOPARPALES |
| IV. GRAPHIDALES | V. COLLEMALES |
| VI. PELTIGERALES | VII. ECTOLECHIALES |
| VIII. CLADONIALES | IX. PARMELIALES |

In this arrangement the orders which are, generally speaking, the more highly developed, are placed last, but in their further consideration it will be more convenient to treat them in the inverse order. The diagnoses of these orders have been given previously (p. 7).

Order PARMELIALES

Family TELOSCHISTACEAE

Characters as defined by Zahlbruckner (38), except that the ascus should be given as usually 8-spored and the spores as usually polari-locular.

Thallus fruticose.

Medulla of loose tissue	<i>Teloschistes</i>
Medulla of closely-packed hyphae	<i>Lethariopsis</i>

Thallus ± foliose; horizontal or partly ascending	...	<i>Xanthoria</i>
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Teloschistes includes some species in which the spores have a similar structure to the rest, but extra lumina have been developed, so that the spore appears to be 3-septate. These have been placed by Zahlbruckner under the sub-genus *Niorma*. Their spores differ from those of the typical *Teloschistes* in a similar way as those of *Callophisma tetrastichum* differ from those of *C. ochraceum*.

Family CALOPLACACEAE

Characters as given by Zahlbruckner (36).

Ascus 8-spored. Thallus and apothecia usually K + purple

Thallus squamulose (usually placodioid) ... *Placodium*

Thallus entirely crustaceous

Apothecia normally yellowish or reddish ... *Callopisma*

Apothecia dark-coloured, K - ... *Pyrenodesmia*

Ascus many-spored. Thallus and apothecia K -

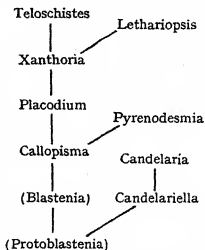
Thallus squamulose ... *Candelaria*

Thallus crustaceous ... *Candelariella*

Placodium is used here in the same way as Lorrain Smith uses *Euplacodium*. Like so many other names of lichen genera it owes its name to vegetative characters. It was first used to denote those lichens having a radiate-squamulose (placodioid) thallus and included some plants in which the spores were not polarilocular. In consequence it has been rejected by some lichenologists, whilst some use it for the placodioid lichens with simple spores. *Amphiloma* and *Gasparrinia* are substituted for it by some authors, whilst others extend its range, so as to include the polarilocular-spored lichens with an entirely crustaceous thallus. Zahlbruckner (36) and Malme (22) use *Caloplaca* and *Callopisma* respectively, in the last-mentioned way. Questions of priority are difficult to settle in such cases but *Placodium* is such a suitable name that its disuse should be deprecated. Its extension to the species which are not squamulose-radiate cannot, however, be justified on account of the appropriateness of the name.

Callopisma includes the sub-genera *Callopisma* and *Blastenia* of Lorrain Smith (32b) for reasons previously given (*vide supra*, p. 13). It includes *Protoblastenia* and *Blastenia* of Zahlbruckner (36) as well as those species of *Caloplaca* in which the thallus is entirely crustaceous. *Protoblastenia rupestris* (Scop.) Zahl. is *Placodium rupestre* B. and R., and is similar to some of the other species of *Callopisma* except in regard to its simple spores. Its inclusion in *Callopisma* is analogous to Zahlbruckner's inclusion of the simple-spored *Lecanora fulgens* Ach. in *Caloplaca* (= *Placodium*). *Lecidea immersa* (Web.) Ach. is put by Zahlbruckner with *Protoblastenia* but seems better kept under *Lecidea*. The reddish or yellowish colour of the apothecium is such a pronounced character in *Callopisma* that the segregation of *Pyrenodesmia*, to contain the species with dark apothecia, seems justifiable, though the old apothecia in some undoubted species of *Callopisma* may become darker. Some parietin is present in *P. variabilis* Krb., but it is absent or doubtful in other species.

Candelaria concolor is placed by Zahlbruckner and Lorrain Smith in the Parmeliaceae, but its affinities seem to be with *Placodium* (see p. 17) or with *Xanthoria lychnea*, states of which it "closely resembles and with which it has been often confounded" (6) p. 368). The genus *Candelaria* should include *Lecanora crenata* Nyl., as this also has its thallus devoid of parietin and its ascus many-spored, though its thallus is more placodioid than that of *C. concolor*. The inclusion of both *concolor* and *crenata* in the same genus can be justified by the consideration of *Lecanora elegans* Ach. and *L. lobulata*



Somm.¹ These two plants differ in thalline characters, more than *Candelaria concolor* differs from *C. crenata*, yet they are included in the same genus by most lichenologists. They are placed as *Placodium elegans* and *P. lobulatum* by Lorrain Smith and as *Caloplaca elegans* and *C. lobulatum* by Zahlbruckner. The close likeness which *Candelaria concolor* exhibits to states of *Xanthoria lychnea* is due to parallel development of the thallus in their ancestral forms of *Candelariella* and *Callopusma* respectively.

The presence of parietin is such a normal feature of the family that it may almost be considered as one of its important characters. In the otherwise abnormal genera, *Candelaria* and *Candelariella*, it is always absent. It is absent in *Placodium medians* Nyl., and its absence may be the reason why Lorrain Smith has named this *Candelariella medians*. This species is very similar to *P. murorum* except in its lack of parietin. If *Candelariella* is allowed to include placodioid species there seems to be no reason why it should be segregated from *Candelaria*. Both genera lack parietin and have many spores in the ascus. *Candelaria* is squamulose whilst *Cande-*

¹ Lyngé, in his recently published *Lich. Nov. Zemlaya*, has rejected this name and substituted *Caloplaca marina*.

lariella is truly crustaceous in its common and original species, *C. vitellina*. If it is desirable to separate the 8-spored species without parietin from *Placodium* and *Callopisma*, *Candelariella* may be extended to include those which are crustaceous, whilst a similar course may be followed for *Candelaria* in regard to the squamulose species. This does not seem desirable and *Candelariella* should be restricted to crustaceous species containing many spores in the ascus. When used in this restricted sense it occupies a similar position to *Callopisma* as *Candelaria* does to *Placodium*. It seems better to retain the name *Placodium medians*, to put *Lecanora crenata* Nyl. under *Candelaria* and *L. epixantha* Nyl. under *Callopisma*. The absence of parietin is to be noted in other plants placed under *Placodium* and *Callopisma*. It is absent in the apothecia of *C. refellens* and in the thallus of several other species.

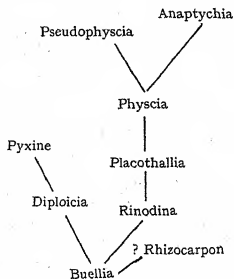
Both Smith and Zahlbruckner recognise the relationship of *Candelariella* to Caloplacaceae though the latter puts the genus in the Lecanoraceae. He, however, says that its inclusion with *Lecanora* is doubtful, as it "zeigt zweifellos Beziehungen zur Gattung *Caloplaca* und ist entweder der Ausgangspunkt der letzteren oder eine reduzierte Form derselben. Ein Zusammenfassen der Gattung, lediglich nach der Sporenform, mit *Lecanora* oder *Lecania*, von welchen sie einzeln genommen allerdings durch geringe, in ihrer Gesamtheit jedoch bemerkenswerte Merkmale abweicht, würde den phylogenetischen Verhältnissen kaum entsprechen" ((36) p. 207). Lorrain Smith also states that "there is also affinity with the genus *Candelaria*" (321).

The two polar loculi are usually evident in the spore but are sometimes absent or indistinct, as in *Placodium fulgens*, *Callopisma nivale*, and *C. rupestre* (*Protoblastenia* r.). A 1-septate appearance may be shown in the spores of *C. luteo-album* and *C. nivale*, whilst *C. tetrastichum* has the spore-contents so arranged as to give a 3-septate appearance. *C. cerinellum* often has more than eight spores in the ascus, but, like the other exceptions, should be considered as a variant within the genus.

Family PHYSCIACEAE

Thallus fruticose, foliose, or crustaceous, heteromerous, with or without rhizinae, with green algal cells. Apothecia orbicular, immersed or sessile, lecideine or lecanorine; paraphyses simple or little branched. Spores usually eight in the ascus, brown, 1-septate, occasionally with further transverse or longitudinal septa; spore-wall usually thick. This family includes Zahlbruckner's Buelliaceae.

There seem to be sufficient grounds for recognising Hue's *Pseudophyscia* (15) as a genus. The fibrous structure of its upper cortex separates it from the plectenchymatous *Physcia*, with which it agrees in the horizontal arrangement of its lobes. It agrees with *Anaptychia* (*sensu stricto*) in the fibrous character of the upper cortex, but its lobes are neither ascending nor fruticose. The inclusion of *Pyxine* has been commented on earlier (p. 12) as an example of the inclusion of lichens with lecideine apothecia in the same family as those with lecanorine. *Placothallia* and *Diploicia* are allowed generic rank. In *Rinodina insperata* the polarilocular character of the spore is very evident and emphasises the close relationship of this family with Teloschistaceae and Caloplacaceae. The tendency of *R. conradi* and *R. diplinthia* to form additional septa in their spores has been previously alluded to (p. 10).



Apothecia lecanorine.

Thallus foliose or fruticose.

Cortical hyphae parallel to the surface of the thallus *Physcia*

Cortical hyphae perpendicular to the surface of thallus.

Thallus fruticose or sub-fruticose *Anaptychia*

Thallus horizontal *Pseudophyscia*

Thallus placodioid *Placothallia* (*Dimelaena*)

Thallus crustaceous *Rinodina*

Apothecia lecideine.

Thallus foliose *Pyxine*

Thallus placodioid or squamulose *Diploicia*

Thallus crustaceous *Buellia*

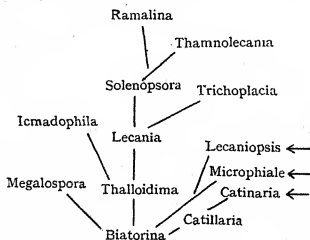
Family RAMALINACEAE

Thallus fruticose with radiate structure and algal cells belonging to Chlorophyceae, medulla webbed, cortical hyphae chiefly arranged longitudinally. Apothecia terminal or lateral, marginate; spores eight in the ascus, colourless, 1-septate.

Ramalina is usually placed with Usneaceae, but is separated from it because of its septate spores.

Family LECANIACEAE

Thallus crustaceous, sometimes placodioid or \pm squamulose, corticate or non-corticate above, attached by hyphae to the substratum, with heteromorous structure and green algal cells, exceptionally orange or reddish. Apothecia usually superficial and sessile, with or without a thalline margin; spores eight or fewer in the ascus (exceptionally more), colourless, 1-septate, occasionally with three septa.



Apothecium with a thalline margin.

Thallus with a dwarf fruticose habit *Thamnolecania*

Thallus placodioid or squamulose *Solenopsora*

Thallus crustaceous.

Apothecium often somewhat stalked; spores ellipsoid and spermatia pleurogenous *Icmadophila*

Apothecium sessile; spores fusiform; spermatia acrogenous *Lecania*

Apothecium without a thalline margin.

Algal cells orange or yellowish.

Algal cells *Trentepohlia*. On bark or mosses *Microphiale*

Algal cells *Phycopeltis*. On leaves *Lecaniopsis*

The arrows in the diagram indicate that a trentepohlioid alga has displaced the original one.

Algal cells green.

Thallus \pm squamulose *Thalloidima*

Thallus crustaceous.

Spores large (over 40μ long) with thick walls; spermatia pleurogenous *Megalospora*

Spores under 30μ with thin walls; spermatia acrogenous.

Apothecium dark; hypothecium usually

dark *Catillaria*

Apothecium and hypothecium pale or

bright coloured *Biatorina*

Thamnolecania, which is given as a sub-genus by Zahlbruckner (36), has a dwarf fruticose habit and partly links up the family to Ramalinaceae. *Solenospora* (*Placodium candicans* Dub., etc.) is synonymous with *Diphvatoria* and *Placolecania*. The presence of 16 or more spores in the ascus of *Lecania syringeae* is too occasional an occurrence to justify its separation from the other *Lecania* species with 1-3-septate spores. *L. vallata* (Stir.) Müll., if placed in this genus, is an aberrant member, as its spores are 8- or more-celled.

Callophis, which is given by Zahlbruckner as a sub-genus of *Physcidia*, may belong to this family but the structure of its spore is doubtful. It is 2-celled and possibly polarilocular. If the latter is the case it is allied to *Placodium*. In any case the broadly-fusiform and 2-celled spore separates the plant from *Physcidia*, with acicular and many-septate spores.

Trichoplacia with 2-septate spores and a squamulose thallus having rhizoids beneath, whilst algal cells are present beneath the apothecium, seems near to *Solenospora* (*Placolecania*), though it is placed as a doubtful member of Phyllopsoraceae by Zahlbruckner and considered to be a fungus by Müller (36) p. 94).

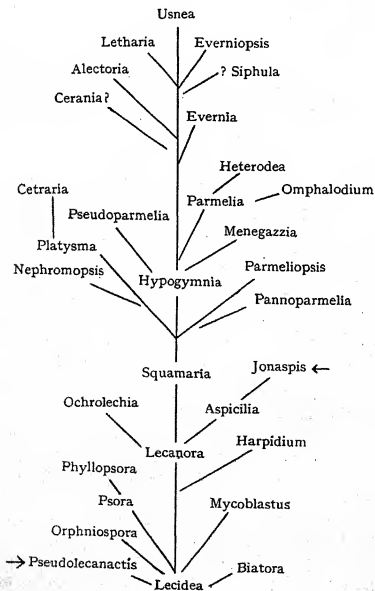
Lecaniopsis is included in Gyalectaceae by Zahlbruckner but it differs from some *Biatorinas* and *Microphiale* by algal characters only.

Family USNEACEAE

Characters as given in Zahlbruckner except that "sporen... zweizellig oder mauerartig-vielzellig," should be omitted. *Ramalina* with 1-septate spores is placed in Ramalinaceae whilst *Oropogon*, with muriform spores, is put under Bacidiaceae. The other genera, *Usnea*, *Letharia*, *Alectoria*, *Evernia*, *Everniopsis*, *Dactylina*, *Dufourea*, *Thamnolia* (*Cerania*), *Siphula* and *Endocena*, may be included in the family, though the three last named are doubtful members, since

their apothecia are unknown or doubtful. Massalongo and Th. Fries considered *Cerania* to have apothecia similar to *Cladonia* and therefore it has been placed in Cladoniaceae by some authors. Harmand⁽¹³⁾ and Crombie⁽⁶⁾ place *Cerania* and *Siphula* together as a family (tribe) of their own. The descriptions of the apothecia remain unconfirmed, and therefore *Cerania* is included with *Siphula* and *Endocena* in Usneaceae, but as of doubtful place.

The spermatia in *Usnea* and *Dufourea* are acrogenous, whilst in the other genera of Usneaceae they are pleurogenous or unknown. There is a possibility that these two genera have evolved from *Parmeliopsis*. In that case they would constitute the family Usneaceae, the remaining genera forming another family.



Family PARMELIACEAE

Characters as given by Zahlbruckner (36) or Smith (32b). *Candelaria*, *Megalospora* and *Physcidia* are included in this family by Zahlbruckner. Reasons for the omission of *Candelaria* have already been given (*vide supra*). *Megalospora* and *Physcidia* should be excluded on account of their septate and acicular spores.

Cetraria and *Platysma* are closely akin, otherwise the radiate structure and habit of the former is nearer to *Usneaceae*. The subgenera *Hypogymnia*, *Menegazzia* and *Omphalodium* of *Parmelia* may be allowed generic rank as they are sufficiently distinct from the other members of this large genus.

The genera of Parmeliaceae are as follow: *Hypogymnia*, *Menegazzia*, *Omphalodium*, *Parmelia*, *Pseudoparmelia*, *Parmeliopsis*, *Pannoparmelia*, *Cetraria*, *Platysma*, *Nephromopsis* and *Heterodea*.

Anzia, with many spores in the ascus, is included in this family by Zahlbruckner, but Reinke puts it with the *Acarosporaceae*. *Pannoparmelia*, placed under it as a sub-genus, should retain the generic rank which Darbishire gave to it.

Family LECANORACEAE

Characters as given by Lorrain Smith (32b) except that the last sentence should read as follows: spores usually 8, rarely more in the ascus, colourless, simple.

Harpidium is placed here by Zahlbruckner. Its spores are simple and it conforms in many other respects, but its pseudoparenchymatous homoiomerous thallus is different from any other member of the family. Reasons for limiting the family are given previously. *Jonaspis* is included for reasons mentioned on p. 17.

Thallus homoiomerous	<i>Harpidium</i>
Thallus heteromerous.					

Thallus squamulose	<i>Squamaria</i>
Thallus crustaceous or almost so.					

Apothecia immersed at first and often remaining so.

Algal cells green	<i>Aspicilia</i>
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Algal cells reddish or yellow	<i>Jonaspis</i>
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Apothecia superficial, or almost so, from the beginning.

Spores very large with thick episore	<i>Ochrolechia</i>
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Spores smaller (less than 40 μ)	<i>Lecanora</i>
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Family LECIDEACEAE

Thallus crustaceous, occasionally squamulose, sometimes evanescent, heteromerous, not rhizinose below; algal cells green. Apothecia circular, discoid or patellate, without a thalline margin: spores usually eight in the ascus, simple and usually colourless.

Spores 1-3-nae, large (over 50μ) with thick walls	<i>Mycoblastus</i>
Spores usually eight, smaller (under 40μ) with thin walls.	
Spores brown, small, more or less spherical ...	<i>Orphniospora</i>
Spores normally colourless.	
Thallus almost foliose	<i>Phyllopsora</i>
Thallus squamulose	<i>Psora</i>
Thallus crustaceous.	
Apothecia dark; hypothecium often dark...	<i>Lecidea</i>
Apothecia pale or coloured; hypothecium often pale	<i>Biatorea</i>

Phyllopsora sometimes differs from other members of the family in its possession of rhizinae.

Uncertainty sometimes occurs as to whether a species should be put in *Biatorea* or *Lecidea* but this slight confusion is compensated for in the reduction of the inconveniently large genus *Lecidea*.

Müller-Argau (26) p. 351 gives good reasons for rejecting the genera founded on the colour of the apothecium. Mudd also is against the use of *Biatorea* as a genus. He says (25) (p. 192), "I see no permanent and definite characteristic distinction between... *Biatorea* and *Lecidea*.... The colour of the apothecium in many instances furnishes a prominent specific character, but it is too variable and too liable to be changed by atmospheric influence to be admitted as a generic guide. The degree of solidity or compactness of the hypothecia, and their different shades of colour, are also too fine and delicate points for generic purposes in the present tribe." These expressions of opinion can easily be justified by examples, but for determinative purposes it is very convenient to have *Biatorea* separated from *Lecidea*.

Family BACIDIACEAE

Thallus usually crustaceous, occasionally squamose, rarely cylindrical, sometimes evanescent, heteromerous, neither corticate nor rhizinose below; algal cells green. Apothecia circular, discoid or patellate, with or without a thalline margin: spores usually eight in

the ascus, usually colourless, with three or more transverse septa, sometimes with longitudinal septa also.

Thallus cylindrical. Spores 1-nae, large and muriform *Oropogon*

Thallus foliose or squamose or squamulose.

Thallus foliose. Apothecia pseudobiotrine and spores acicular, 3-septate *Megalospora*

Thallus somewhat foliose or squamose.

Apothecia lecanorine. Spores acicular, many-septate *Physcidia*

Apothecia lecideine. Spores 3-15-septate *Psorella*

Thallus squamulose. Apothecia lecideoid and spores 3- or more-septate *Tominia*

Thallus crustaceous (not or very little squamulose)

Algae cells orange or reddish *Lecanactis*

Algae cells green.

Apothecia coloured and with thalline margin; hypothecium pale.

Thallus usually corticate. Spores 8-nae, colourless, 3- or more-septate *Haematomma*

Thallus not corticate. Spores 1-nae, muriform *Myxodictyon*

Apothecia without thalline margin, coloured or dark. Spores usually eight.

Spores with transverse septa only, usually colourless.

Spores 3- or more-septate, fusiform, colourless *Bilimbia*

Spores pluriseptate, elongate-acicular, colourless *Bacidia*

Spores 5-10-septate, very large, often 1-nae *Bombyliospora*

Spores 3-septate, brown. Parasitic *Leciographa*

Spores muriform, colourless or brown.

Spores usually 8-nae and halonate; paraphyses branched *Rhizocarpon*

Spores usually 1-nae, not halonate; paraphyses unbranched *Lopadium*

Oropogon is similar to *Alectoria* and is often placed in *Usneaceae*. Its position depends upon its origin. If the septa have developed in the spore since the fungus took on a partnership with the alga, its affinity is with *Alectoria*. If the spores of the original fungal partner

were septate, *Oropogon* is allied to *Myxodictyon*. The large muriform and sometimes brown spore which is single in the ascus suggests affinity with *Myxodictyon*, but there are no certain links, in regard to thalline development, connecting the two genera.

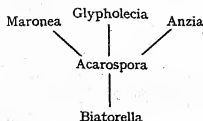
Megalospora, because of its acicular spores which may become 3-septate, is transferred from Parmeliaceae.

Psorella is like a foliose or squamose *Bilimbia* or *Bacidia* and may differ from other members of the family in possessing rhizinae. Zahlbruckner placed it in the family Phyllopsoraceae with *Phyllopsora* and the doubtful *Trichoplasia*. *Phyllopsora*, as its name implies, is similar to a foliose *Psora* and is here included in Lecideaceae, whilst *Trichoplasia* is here regarded as akin to *Solenopsora*. The family Phyllopsoraceae therefore disappears, the three genera which constituted it being distributed to three different families.

Some species of *Rhizocarpon* appear to be related to *Buellia*. *R. alboatrum* is usually quite distinct from *B. myriocarpa*, but occasionally there is some difficulty in distinguishing them. In both the paraphyses are capitate and dark-tipped. The spores become dark in both plants, but in the former become muriform, whereas in the latter the septation does not proceed further than the development of one transverse septum. In the formation of the spores *R. alboatrum* partially "recapitulates" the mature *B. myriocarpa*. The spores are 1-septate, become dark, and then extra transverse and longitudinal divisions occur. In *R. oederi* the septation seldom proceeds further than the development of two additional transverse septa. There are some reasons for regarding both *Rhizocarpon* and *Bilimbia* as mixed, i.e. as having species relegated to them which have had an origin different from the others.

Family ACAROSPORACEAE

Characters as given by Zahlbruckner. He includes *Thelocarpon* but this seems to be better placed in a family of its own (Thelocarpaceae) amongst the Pyrenocarpales, a course followed by Lorrain Smith (32a), on account of its perithecium. *Maronea* may



have a septum developed in the spore, but otherwise agrees with the other genera (simple-spored) of the family.

Anzia has a many-spored ascus, but is excluded from the family by Zahlbruckner and placed in Parmeliaceae. Its affinities seem to be nearer to Acarosporaceae, in which it was placed by Reinke (30).

Family PERTUSARIACEAE

Characters and genera as given by Zahlbruckner. *Perforaria* has the disc so little exposed that the thecium is almost a perithecium. *Varicellaria* has the spore 1-septate but, in other respects, is so much like a *Pertusaria* that a phylogenetic connection is probable.

Family GYROPHORACEAE

Characters as given by Zahlbruckner ((36) p. 209). This seems like a natural family but there are many deviations from what may be considered as a typical member. Most species of *Gyrophora* have a dark foliose thallus attached by a central hold-fast, furrowed apothecia (gyrose) and 8-nae, simple and colourless spores. Even in the genus *Gyrophora* there is considerable diversity. Some species (e.g. *G. cylindrica*) have a divided or lobed thallus attached by rhizinae to the substratum, and in some others (e.g. *G. leiocarpa*) the apothecium is not furrowed. The other genera of the family agree with the typical Gyrophoras in general appearance and in their manner of attachment but the spores are septate. They are 1-septate and colourless in *Charcotia*, 1-septate and brown in *Dermatiscum* and muriform in *Umbilicaria*, the latter also having fewer spores in the ascus. The apothecial disc is usually plane except in *Gyrophora*. The only distinguishing characters common to all the genera are vegetative ones (which are possessed by *Dermatocarpon minutum*) and it is possible that their apparent relationship is due to convergence, rather than to phylogenetic connection.

Family THELOTREMACEAE

Characters as given by Lorrain Smith ((32b) p. 378). The two families Thelotremaceae and Diploschistaceae scarcely differ except that the latter has green algal cells. The relative positions of these families to others, as given by Zahlbruckner (36), indicate their affinity with the Ectolechiales. The thallus is usually primitive and sometimes homoiomerous but is heteromerous in the higher-evolved members, so that the family may be considered as a low member of the Parmeliales.

Phlyctis, *Phlyctella*, and *Phlyctidia*, though placed by Zahlbruckner in Lecanoraceae, show better kinship with this family, in which *Phlyctis* is placed by Lorrain Smith.

Conotrema has such peculiar spores that its inclusion in the family is doubtful.

Order CLADONIALES

Characters as given on pp. 6 and 7, or as defined by Lorrain Smith for Cladoniaceae (32b) p. 402). This order may be arranged in three families: (1) Cladoniaceae; with simple spores; (2) Stereocaulonaceae, with spores 3- or more-septate; and (3) Gomphillaceae, consisting of the monotypic genus *Gomphillus*, which differs in so many respects from the members of the other families that it is best placed in a family of its own.

This order is sometimes considered to be of monophyletic origin, in which there have been "changes in form and septation (of the spore) not commensurate with thalline advance" as "in *Gomphillus*, with primitive thallus and podetium, the spores are long and narrow with about 100 divisions" (32c) p. 293). The three families, however, may have had distinct origins, and the similarity in podetial development may be due to convergence.

Family CLADONIACEAE

The spores are usually simple but occasionally 1- or more-septa are developed in *Pycnothelia*, *Thysanothecium* and *Baeomyces*. In some species of *Baeomyces* the podetium originates in the same way as an apothecial stalk whilst in others it is formed by the extension upwards of the primary granule. The latter method of podetial development also occurs in *Pilophorus*.

Podetia usually well developed and often widening upwards.

Primary thallus squamulose and \pm persistent;	
podetia often scyphiferous	<i>Cladonia</i>
Primary thallus evanescent or obsolete.	Podetia rarely scyphiferous.
Podetia perforated by many pores ...	<i>Clathrina</i>
Podetia not perforated	<i>Cladina</i>
Primary thallus crustaceous and persistent;	
podetia short and not scyphiferous	<i>Pycnothelia</i>
Podetia short and not widening upwards.	
Podetia clothed with granules or squamules	<i>Pilophorus</i>
Podetia not clothed with granules or squamules.	
Primary thallus foliose with marginal podetia	<i>Gymmoderma</i>

Primary thallus crustaceo-granulose or squamulose with superficial podetia ...	<i>Baeomyces</i>
Primary thallus lobed and leaf-like above	<i>Thysanothecium</i>

Family STEREOCAULONACEAE

Podetia usually well developed, solid, clothed with granules or squamules.

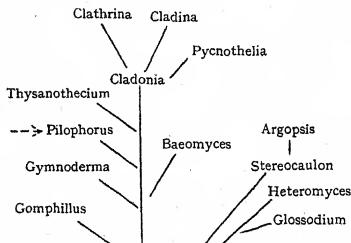
Spores elongate, 3- to many-septate ... *Stereocaulon*

Spores muriform *Argopsis*

Podetia short, \pm hollow and naked. Spores 1-3-septate.

Primary thallus foliose. Spores cylindrical *Heteromyces*

Primary thallus crustaceous. Podetia widened above with apothecia on one side. Spores fusiform *Glossodium*



Family GOMPHILLACEAE

Gomphillus, the only member of the family, has a superficial and naked podetium bearing 1-5 apothecia at the apex. In many other respects it differs from other members of the Cladoniales. Its thallus is a thin gelatinised membranous crust and homoiomerous. Its spores are remarkable for their long filiform and many-celled (up to 100) character. Despite the homoiomerous thallus, the presence of podetia justify its inclusion in the *Cladonia* group.

Order ECTOLECHIALES

In this order the thallus is primitive (usually homoiomerous) and the apothecial wall is absent or poorly developed. It includes the families Ectolechiaceae, Byssolomaceae, Coenogoniaceae, and Chrysothricaceae. Ectolechiaceae has many-septate or muriform

spores. The genera *Asterothyrium*, *Gonolecania*, *Byssolecania* and *Actinoplaca* have spores which are 1-2-septate, and a separate family, *Asterothyriaceae*, may be formed to receive them. The family *Gyalectaceae* (characters as given by Lorrain Smith) is also better included in the *Ectolechiales*. Zahlbruckner includes in the family the simple-spored *Jonaspis* and the 1-septate-spored *Microphiale* and *Lecaniopsis*. The relations of these have been previously indicated (*vide supra*, pp. 16, 17, 23, 24, 26). *Ramonia* is of doubtful position. The following key shows the relationships of the members remaining in *Gyalectaceae*:

Thallus with <i>Scytonema</i> algae	<i>Petractis</i>
Thallus with <i>Phycopeltis</i> algae	<i>Semigyalecta</i>
Thallus with <i>Trentepohlia</i> algae.				
Ascus with 12 or more 3- or more-septate spores				<i>Pachyphiale</i>
Ascus with 8 or fewer spores.				
Apothecium and hypothecium dark	<i>Sagiolechia</i>
Apothecia coloured or pale	<i>Gyalecta</i>

Order PELTIGERALES

This order contains the families *Peltigeraceae*, *Stictaceae* and *Pannariaceae* (*vide supra*, pp. 7, 8).

The characters of these families, as arranged by Zahlbruckner and Lorrain Smith, are generally accepted. Zahlbruckner includes plants having blue-green algae in the genus *Nephroma*. It seems preferable to keep *Nephroma* for those plants possessing green algae in their thalli, and to put those possessing *Nostoc* in *Nephromium*, which Zahlbruckner uses in a sub-generic manner. A similar rearrangement, or rather a return to the Nylanderian arrangement, also seems preferable in regard to *Sticta* and *Stictina*, or *Lobaria* and *Lobarina*, for exactly the same reason. Lorrain Smith is not consistent when she accepts *Nephroma* and *Nephromium* as two genera because of the different alga present in the thallus, but includes *Stictina* with *Sticta* and *Lobarina* with *Lobaria*. In a similar way, Nylander, who was followed by many other lichenologists, used *Peltidea* and *Peltigera*, the former containing those species of *Peltigera* (*sensu lato*) in which the algal layer of the thallus was green, whilst *Peltigera* was reserved for the ecephalodiiferous species with a blue-green algal layer. These names were in vogue for many years but, as the Acharian name of *Peltidea* was used as a synonym of *Peltigera* (*sensu lato*), it is difficult to reconcile them with the Vienna

rules unless *Peltidea*, as used by Nylander, is accepted as a *nomen conservandum*. The following tabular arrangement shows how Nylander named the genera:

Thallus with green algae	Nephroma	Sticta	Lobaria	Peltidea
Thallus with blue-green algae	Nephromium	Stictina	Lobarina	Peltigera

The position of *Placynthium* also requires to be rendered more definite. Lorrain Smith (32a) p. 31, "on account of the homioimerous thallus," places it in the family Ephebaceae whilst Zahlbruckner (36) retains it in Pannariaceae. The thallus is not quite homioimerous and, in many respects, approaches closely to that of the genus *Parmeliella* (*Pannularia* of Nylander). Nylander actually included our common British species in the genus *Pannularia* as *P. nigrum*. The spores are 1- or more-septate, whilst in most of the genera included by Zahlbruckner in Pannariaceae (*Psoroma*, *Psoromaria*, *Pannaria*, *Parmeliella*, *Erioderma*, *Coccocarpia*, *Lepidocollema*, *Lepidoleptogium*) they are simple. Apart from this septation of the spore, the genus *Placynthium* (as well as the genus *Massalongia*) is better retained in the Pannariaceae. The common British member of the genus, *Placynthium nigrum* Gray (12), varies very much in the septation of the spores. These are usually 1-septate, but in some apothecia (otherwise seemingly well developed) they are mostly simple, whilst other apothecia, on the same rock, possess 3-septate spores.

Hydrothyria is put with Pannariaceae (36) but it seems more at home with *Peltigera*.

Order COLLEMALES

This order (*vide supra*, p. 7) contains the families Heppiaceae, Collemaceae, Physmaceae (to include the genera parallel to those of Collemaceae, but with simple spores), Lichinaceae, Ephebaceae and Pyrenopsidaceae. As algal characters are largely used for the arrangement into families, some slight alterations (other than the one indicated above) may be necessary. The species with definite perithecia should be relegated to the Pyrenocarpaceae. *Latzelia*, because of its muriform spores, is more probably derived from *Collema*, even though its thallus is not (or only very slightly) gelatinous, and contains algal cells like those of *Heppia*.

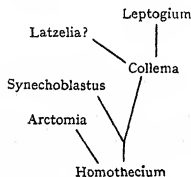
The changes chiefly involve the families Collemaceae and Physmaceae, which are therefore given in more detail.

Family COLLEMACEAE

Thallus gelatinous when moist, homoiomorous, usually \pm foliose, corticated or non-corticated, with or without rhizinae, with blue-green algal cells single or moniliform. Apothecia \pm open, with or without thalline margin, immersed or sessile; spores septate, often muriform. Spermogonia with pleurogenous or acrogenous spermatia.

The characters and the genera are similar to those given by Lorrain Smith (⁽³²⁾ p. 46) or by Zahlbruckner (⁽³⁸⁾ p. 164), except that the genera with definitely closed apothecia, or with simple spores, are excluded. Lorrain Smith is followed in the allowance of generic rank to *Synechoblastus* (including *Collemodiopsis*).

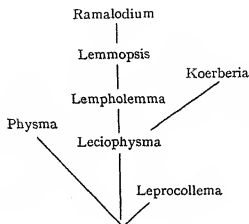
On account of the pseudoparenchymatous nature of the thallus or its cortical portion, the family Leptogiaceae has been separated. Mudd's diagnosis (⁽²⁵⁾ p. 20) is as follows: "Thallus coriaceous,



membranaceous, foliaceous or caulescent, variously lobed and lacinated, rigid when dry, gelatinous and somewhat turgid when wet; epithallus distinctly cellular; gonidiac granules simple or moniliform-coherent. Apothecia scutelliform or patellaeform." Good reasons may be advanced for the retention of this family but the general evidence is against it. The presence of pseudoparenchyma is not a clear-cut character, as in *Synechoblastus nigrescens* Anzi. (*Collema* n. Ach.) and *S. rupestris* A. L. Sm. (*C. flaccidum* Ach.), pseudoparenchyma is absent except in the thallus near the apothecia. There also seems to be a parallel development of pseudoparenchyma in the simple-spored genera placed here in Physmaceae. Just as *Collema* advances in thalline structure to *Leptogium*, so *Lemphollemma* (with simple spores and non-corticated thallus) advances, through some species with pseudoparenchyma near the apothecia, to *Lemmopsis* (with simple spores and corticated thallus).

Family PHYSMACEAE

Thallus \pm gelatinous when moist, crustaceous or foliaceous or minutely fruticose, corticated or non-corticated, with or without rhizinae, with blue-green algal cells. Apothecia \pm open, sometimes apparently almost closed, usually with thalline margin, mostly innate; spores simple. Spermogonia with pleurogenous or acrogenous spermatia.



Spores spherical or \pm ellipsoidal, straight.

Thallus crustaceous, scarcely gelatinous. Apothecia biatorine ...

Leprocollema

Thallus \pm fruticose ...

Ramalodium

Thallus usually small-lobed, foliose or fruticulose, gelatinous.

Thallus corticated ...

Lemmopsis

Thallus not corticated.

Apothecia lecanorine.

Spores thick-walled or with epispore ...

Physma

Spores thin-walled. Thallus usually smaller and more appressed ...

Lempholemma

Apothecia lecideine ...

Leciophysma

Spores acicular and contorted ...

Koerberia

(To be continued)

THE REGENERATION OF THE STEM APEX

BY MARY PILKINGTON

(With Plates I and II and 20 figures in the text)

INTRODUCTION

THE growing point of the stem has been the subject of comparatively little experimental work. Observation of the normal growing point has not led to the solution of any of the problems which it presents, such as its permanently embryonic condition or the factors determining the sequence of the leaf primordia. One of the simplest methods of attacking the problem of apical growth experimentally is that of seeing how the apex responds to an operation or injury such as decapitation or a median split. A number of investigations have been made on the regeneration of the stem apex after these operations but the results are not absolutely conclusive.

Lopriore in 1898 described the regeneration of the stem apex following a longitudinal split. He used *Acer pseudoplatanus*, *Vitis vinifera* and *Helianthus annuus*. About 6 months after the operation the two halves had grown out to a length of 1 m. or more and the wound was found at the base of the fork. The new growing points appeared normal. A similar result was obtained by Karzel (published in 1924 though the experiments dated from 1909) working with *Acer pseudoplatanus*, *Plectranthus fruticosus* and *Bowiea volubilis*. The examination was made about 3 months after the operation. Karzel's observations were mainly confined to the formation of callus and differentiation of new vascular elements at the base of the fork. As no observations were reported on the early stages of regeneration it is not possible to tell how the new growing points were formed in these cases. Another case of regeneration is reported by Kny who obtained a double sunflower head after splitting the young capitulum.

An extensive investigation was made by Linsbauer in 1917. He worked with *Phaseolus coccineus* and *Polygonatum officinale*. He claims to have seen the early stages of regeneration following a longitudinal split, decapitation and a median prick with a needle. Three to five days after the operation he saw in several cases a small hump growing out from the undamaged surface of the original

apex by the side of the wound. This hump he considered to be a new growing point. Only that part of the apical meristem which lay above the youngest leaf primordia gave rise to a new growing point, consequently regeneration rarely took place after decapitation as it depended on the removal of an extremely small piece. A median split was followed by the initiation of a new growth centre in each of the split halves. In no cases did regeneration of the apex take place from the wound surface.

It cannot be said that Linsbauer's evidence is absolutely conclusive as the hump which he considered to be a new apex was examined at such an early stage that its nature could not be determined with certainty. It did not resemble the normal apex in shape and disposition of the tissues nor did it bear any leaf primordia. The presence of lateral members arising in a phyllotactic sequence is the most conclusive evidence of a stem apex. Evidence of this kind Linsbauer obtained in the case of the capitulum of *Helianthus*. Sachs described a sunflower head in which the central cone had been accidentally destroyed with the result that a new circular meristem had arisen around the damaged growth centre producing florets in an inverse order, the oldest being towards the centre of the disc. Linsbauer was able to reproduce this condition by pricking the central cone of a young inflorescence. This together with Kny's experiment shows that true regeneration takes place in the sunflower capitulum, though in the former case the new meristem was circular.

Other experiments on regeneration after decapitation are reported by Reuber working on *Populus nigra*. In five cases the wound surface developed a conical hump which he considered to be a new apex though its contour differed from that of a normal growing point and it bore no leaf primordia. In a single case a normal apex was found but the wound could not be traced. He concluded that regeneration took place as in the root, the new apex growing out from beneath the wound surface. It is obvious that this evidence is inconclusive.

From these investigations it appears extremely probable that regeneration takes place in the manner described by Linsbauer, but as definite proof of regeneration was only obtained in the case of the sunflower capitulum it was considered that a further investigation was necessary.

DESCRIPTION OF EXPERIMENTAL PLANTS

The plants used were *Vicia faba* (races Johnson's Long Pod, Aquadulce Long Pod and Taylor's Broad Windsor) and *Lupinus albus*. These plants were chosen because they both had relatively large apices and could easily be grown throughout the year in a greenhouse. The apices of both the Broad Bean and the Lupin require some description.

The phyllotaxis of *Vicia faba* is a distichous system. A section passing through the apex along the line *ab* in the diagram includes all the leaves and their axillary buds—Fig. 1—while a section along the line *cd* at right angles includes only the stipules (see Pl. I, phot. 1). For purposes of description the line *ab* will be referred to as "the plane of the leaves" and the line *cd* as "the plane of the stipules."

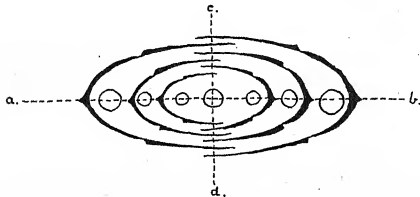
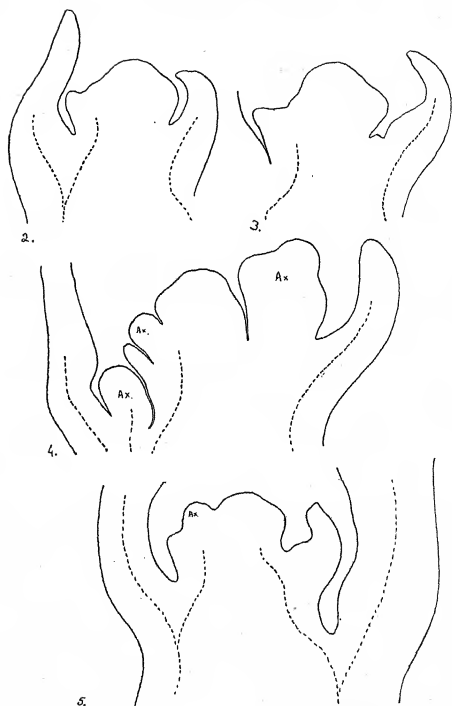


Fig. 1. Diagram of the apex of *Vicia faba*. Showing distichous arrangement of leaves and axillary buds.

The young apex is erect. The primordia giving rise to axillary inflorescence arise at the growing point at a very early stage in the axil of about the eighth leaf. Shortly after the appearance of flower buds the apex begins to bend over in the plane of the stipules, the curvature takes place in the zone of the youngest leaf primordia and increases with age, so that when the seedling is about 6 in. high the apex is completely turned to one side and buried beneath a mass of flower primordia. Consequently it is only possible to cut sections through the apex in the plane of the leaves at a very early stage. All sections made for the purpose of observing regeneration necessarily passed through the plane of the stipules. It was also impossible to decapitate the apex successfully except in the young erect stage.

Very great variation was found between different apices. Figs. 2 to 5 show the apices of two Johnson's and two Aquadulce Long Pods; it is evident that the level of the youngest primordium varies



Figs. 2 to 5. *Vicia faba*. The apex in the plane of the leaves. The dotted lines represent procambial strands and vascular bundles. Ax represents an axillary bud giving rise to an inflorescence. $\times 54$.

Fig. 2. Aquadulce Long Pod. The youngest primordium arises at a longitudinal distance of 60 to 70μ from the tip.

Fig. 3. Aquadulce Long Pod. The youngest primordium arises at a longitudinal distance of 80 to 90μ from the tip.

Fig. 4. Johnson's Long Pod. The level of the youngest primordium is 60 to 70μ from the tip. A rather older stage than the two Aquadulce Long Pods. A large axillary bud is seen to the right of the apex.

Fig. 5. Johnson's Long Pod. The level of the youngest primordium is 45 to 55μ from the tip.

considerably in each case. These differences may partly be accounted for by the fact that an interval elapses—called by some authors the “plastochron”—between the initiation of successive primordia. During this interval the apex performs a definite amount of growth, so that at the end of a plastochron it projects considerably farther beyond the level of the last primordium than at the beginning. According to Priestley the shape of the apex varies with the degree of illumination, and it is possible that this factor may also have been responsible for some of the variations met with.

The phyllotaxis of *Lupinus albus* is a spiral system with 3 + 5 parastichies. The inflorescence in this case is terminal, the flower primordia not appearing until the seedling is about 8 weeks old. The apex at first is only slightly convex, later it rises to a higher dome (Fig. 15); and at the inflorescence stage the dome becomes even more pointed, the tip projecting considerably beyond the youngest lateral primordia.

METHODS

The operations were of three kinds: (1) decapitations, (2) longitudinal splits, (3) pricks. The bud was dissected beneath a binocular dissecting microscope giving a magnification of 30 diameters, and the two former operations were performed with a sharp scalpel, a needle being used for pricks. The leaves or stipules were left undamaged as far as possible in order to protect the apex after the operation. The material was fixed in acetic acid and alcohol and examined by hand sections. Ehrlich's haemotoxylin was used as a stain.

REGENERATION AFTER A MEDIAN LONGITUDINAL SPLIT

When the apex was halved longitudinally regeneration of the two halves nearly always followed. The split was generally about 200μ in length. If it was truly median each half developed a new apex which continued growth normally. If the apex was unevenly split only the larger half regenerated. Negative results were only obtained when the operation had been carelessly performed and the apex considerably damaged. As it was impossible to cut a median section through the apex in the plane of the leaves except at a very early stage of development the split was always made to pass through this plane so that the plants could be examined by sections in the plane of the stipules at right angles to the split.

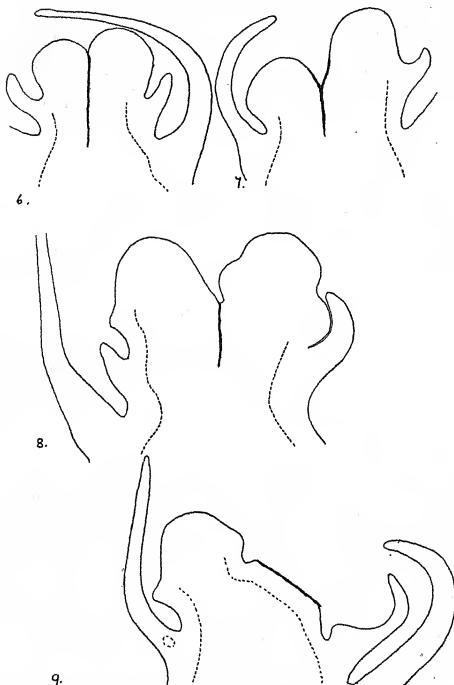
The early stages of regeneration were studied in *Vicia faba*. (As the three races showed no differences in the manner of regeneration

no attempt will be made to distinguish between them.) No. 99 (Fig. 6) and No. 170 (Pl. II, phot. 5) were both examined 7 days after the operations. Two new apices are clearly visible, one on either side of the split. No. 123 (Fig. 8) is a later stage. It is clear that these two new growing points cannot be axillary buds since the whole of the old growing point is used up in the formation of the two new ones. It is also evident that regeneration has not taken place from the wound surface but in the manner described by Linsbauer—through the initiation of two new growth centres on either side of the split. The wound surface was covered with a yellow brown scab the nature of which was not determined; it appeared to be impermeable as the wound was always sterile and the apex never suffered from water loss after the operation. This impermeable layer probably prevented the re-union of the split halves which never took place in *Vicia faba* though the wound surfaces were in contact. A wound meristem was always formed, the new cell walls being parallel to the wound surface, and in this way the diameter of the stem was increased. At a later stage leaf primordia appeared on the new apices and procambial strands were differentiated parallel to the wound surface. Regeneration following a split was recorded in 14 plants of *Vicia faba*.

The early stages of regeneration in *Lupinus albus* were essentially the same as in *Vicia faba*. No. 128 (Fig. 19), examined after 13 days, shows the two new apices growing out and bearing leaves. The two leaves on either side of the cut appear abnormally large in relation to those on the other sides of the growing points. The reason for this exaggerated growth is not known though it was seen in other plants as well.

The Lupin generally produced callus in addition to the wound meristem whereas the Broad Bean produced no callus. This growth of callus from the two adjacent wound surfaces frequently led to the re-union of the two split halves. The union was effected by interlocking callus cells; it only occurred in the lower region of the split and did not extend to the apex, so that the regeneration of the apex was unaffected.

Where a fairly deep cut had been made it could be seen that the differentiation of the procambial strands on the side of the split took place basipetally, the new strand appearing first in the upper region as in No. 72 (Fig. 10) examined after 13 days. The procambial strand is only seen in the upper part of the split, differentiation having not yet taken place in the lower part. The union of the two halves by means of the callus is seen towards the base of the split.



Figs. 6 to 9. *Vicia faba*. Wound surface represented by thick black line. Procambial strands and vascular bundles dotted.

Fig. 6. No. 99. Examined 7 days after a split.

Fig. 7. No. 235. Decapitated and split, examined after 10 days.

Fig. 8. No. 123. Examined 13 days after a split.

Fig. 9. No. 184. Examined 13 days after a decapitation. $\times 54$.

The further development of the two shoots was also followed. No. 73 was split on June 1st and photographed on August 1st (Pl. II, phot. 4). The forking of the main axis as a result of the split

is clearly visible. No. 75 was grown for 5 months after the operation; one shoot reached a length of 64 cm., the other of 55 cm. Both flowered normally and produced lateral branches. The phyllotaxis system was $3 + 5$ as in the normal apex. It is evident that the two split halves of the growing point gave rise to branches which con-

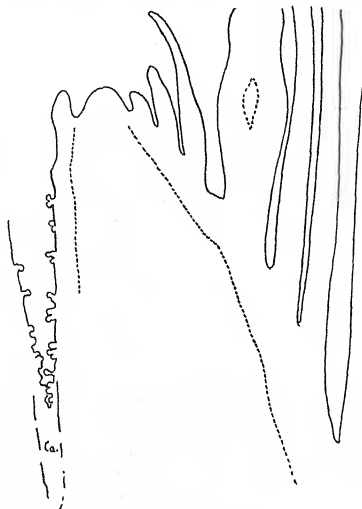


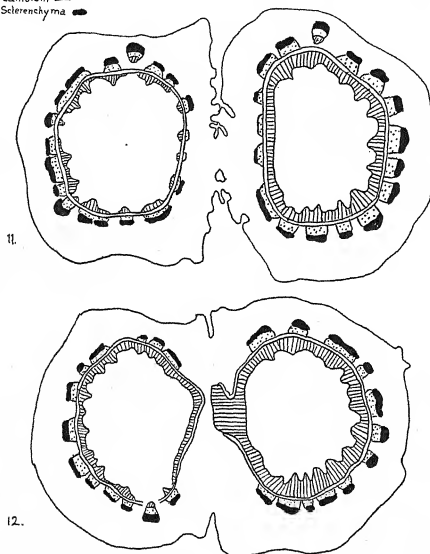
Fig. 10. *Lupinus albus*, No. 72. Examined 13 days after the split. Only one new apex is shown. The wound surface is on the left with callus growing from it. In the lower part of the split the two halves are united by means of the callus, Ca. The new vascular strand has only differentiated in the upper part of the split region.

tinued the normal growth of the terminal shoot. Altogether 21 cases of regeneration following a median split were recorded in the Lupin.

The developments which had taken place at the wound surface were briefly investigated. The scar extended about 2 cm. above the base of the fork. As in *Vicia faba* the wound meristem increased the diameter of the two split halves, and new vascular strands appeared parallel to the wound surface. The xylem, which may be described

as "wound wood," lacked protoxylem groups. The vascular system on the side of the wound was considerably less developed than that of the opposite side of the stem.





Xylem 
 Phloem 
 Cambium 
 Sclerenchyma 

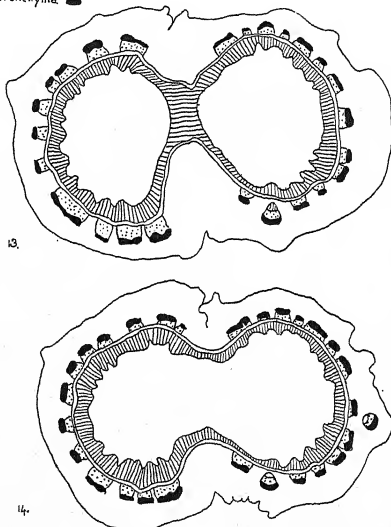


Figs. 11, 12. *Lupinus albus*, No. 39. Transverse sections through the stem at different levels 10 weeks after the operation. Description in text. $\times 22$.

In the lower region of the split where the wound surfaces were in contact re-union was effected by an interlocking callus. This stage is well seen in No. 39 (Fig. 11), examined 10 weeks after the operation. The union in this region is purely cortical and the two steles remain distinct. Below this is a zone where the steles unite. The xylem on the inner sides of the two rings becomes coalescent (Fig. 13), this xylem being also of the nature of wound wood. At a lower level

there is a single stele (Fig. 14); two grooves are seen in the cortex on either side of the stem. Along the line between these grooves the xylem is represented by wound wood. The diameter of the stem has increased considerably at right angles to the split.

Xylem 
 Phloem 
 Cambium 
 Sclerenchyma 



Figs. 13, 14. *Lupinus albus*, No. 39. Transverse sections through the stem at different levels 10 weeks after the operation. Description in text. $\times 22$.

It is uncertain whether the split passed through the region which later developed a single stele, or whether this zone was affected by the overlying split. The manner in which the re-union of the stem and the junction of the steles took place was not investigated further as these problems bear no direct relation to the regeneration of the apex.

REGENERATION AFTER DECAPITATION

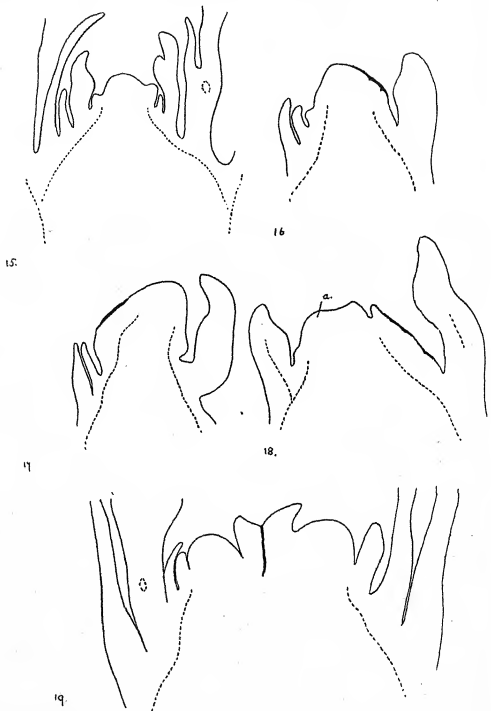
It was far more difficult to obtain a positive result after decapitation since it was found that regeneration never took place if the cut was made below the youngest leaves, and in many cases when the cut was made above this level no regeneration followed.

Experiments with the Lupin will be described first. The apex of the young seedling projects so little that it is almost impossible to remove the tip without including the youngest leaf primordia. It was thought that the highly arched dome of the inflorescence apex would be more suitable for decapitation; but negative results were obtained in this case also, though the cut was made well above the youngest lateral members. This point will be referred to again later.

A positive result was finally obtained when the apex was decapitated at a stage intermediate between the two above described. A small hump was seen growing out by the side of the wound. No. 89 (Fig. 16), examined after 10 days, shows this stage. No. 50 (Fig. 17) has grown further. This hump is clearly a new apex since it has assumed a terminal position and continues the growth of the stem. The wound is now in an oblique position and the procambial strand has differentiated beneath the wound surface. The new growing point was seen to bear leaf primordia after 15 days in No. 105 (Fig. 18). It may be concluded that after decapitation the new apex grew out from the surface of the original meristem above the youngest leaves. In no case did regeneration take place from the wound surface.

Regeneration was observed in 15 cases in the Lupin following decapitation. In 8 of these the examination was made after 3-4 weeks, and in every case the new apex was continuing the normal growth of the stem. The wound either appeared as a slight scar among the leaf bases some distance from the tip, or it could not be recognised.

Decapitation of the apex of *Vicia faba* was also a difficult operation; regeneration was only obtained after very numerous negative results. The erect apex of the young seedling can be more easily decapitated than that of the older seedling, in which the apex is turned to one side. Regeneration took place in the same manner as in the Lupin. After 4 days the new growing point could be seen growing out from the surface of the original apex—see Fig. 20, No. 273. No. 178, examined after 10 days, shows a later stage (Pl. I, phot. 2). The wound is visible on the right; the wound



Figs. 15 to 19. *Lupinus albus*. The wound is represented by a heavy black line. Vascular bundles and procambial strands dotted. $\times 54$.

Fig. 15. The normal apex of a seedling about 4 weeks old.

Fig. 16. No. 89. Ten days after being decapitated. The new apex is visible as a slight hump to the left of the wound.

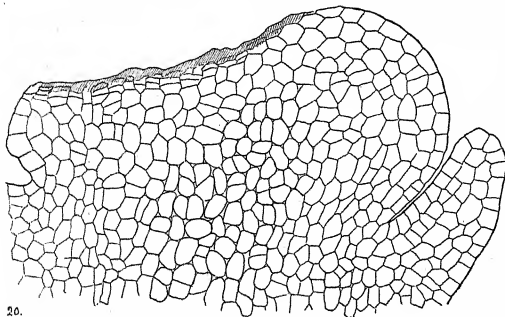
Fig. 17. No. 50. Ten days after being decapitated.

Fig. 18. No. 105. Fifteen days after being decapitated. The new apex *a* bearing leaf primordia is seen to the left of the wound. It has a flatter dome than the two above.

Fig. 19. No. 128. Thirteen days after being split. Description in text.

meristem producing cell rows at right angles to the wound surface can also be distinguished. A later stage, in which the apex bears leaf primordia, is shown in No. 184 (Fig. 9), examined after 13 days.

It might be suggested that the new growing point was really an axillary bud arising in the axil of one of the uppermost leaves. There is evidence that this is not the case, since in *Vicia faba* the new apex nearly always grows out in the plane of the stipules and not in the axil of the leaves. In those cases where it appeared in the same plane as the leaves it was far more difficult to distinguish it from an axillary bud in the early stages. But at a later stage it could



20.

Fig. 20. *Vicia faba*, No. 273. Four days after a decapitation. Wound surface shaded. The new apex is seen to the right of the wound. $\times 185$ approx.

not be confused, because the axillary buds of all but the first six or eight leaves give rise to flower buds only, whereas the new apex continues the growth of the main axis.

An attempt was made to determine what was the largest piece that could be removed from the apex of *Vicia faba* without destroying its powers of regeneration. Measurement of the decapitated piece involved considerable difficulty. The method finally adopted was as follows. The scalpel with the tip adhering to its flat surface was transferred to the stage of the microscope and placed with the edge of the blade vertical so that the depth of the adhering apex could be measured. The measurement was made as rapidly as possible to avoid shrinkage owing to water loss. Another method sometimes

used was that of mounting the tip in glycerine on a slide and measuring the transverse diameter. The length corresponding to the diameter was measured on a longitudinal section of the normal apex. The results are tabulated below. It was not found possible to measure more accurately than to within the nearest 10μ .

Length of piece removed from apex μ	Number of cases in which regeneration occurred	Number of cases which failed to regenerate
40-50	6	1
45-55	2	0
50-60	4	4
55-65	3	1
60-70	3	5
70-80	3	2
80-90	0	1
90-100	0	2

The reason why there are so few cases in which 80-100 μ was removed was because very few apices were found in which more than 80 μ could be removed without including the youngest primordia. Variations in the apices of different plants may account for the variability of these results. Measurements of different apices showed that the distance of the youngest primordium from the tip varied from 50 to 90 μ (see Figs. 2 to 5). It was not considered worth while to continue these measurements since a consistent result could hardly be expected, and in any case the figures obtained could only apply to *Vicia faba*.

It was found that the new apex generally grew out above the youngest stipules and at right angles to the leaves. Regeneration in the plane of the leaves only took place in a few cases in which a very small piece had been removed, such as Nos. 178 and 217, in which the piece measured 40 to 50 μ . As the youngest primordia do not occupy the whole circumference of the apex there is a space on the stipular sides from which a new apex could grow out even if the cut reached to the level of the youngest leaves. At a slightly lower level this space is occupied by the young stipules, which grow out rather later than the leaves to which they belong and extend half-way round the axis on each side.

It is probable that not only that part of the growing point which lies above the youngest primordia is capable of giving rise to a new apex, but any undamaged superficial area of the meristem either above or between the youngest primordia. Whether or not regeneration takes place probably depends on the presence of a sufficiently large surface area from which the new apex can develop. It appears

that these conditions are never fulfilled when more than 80μ is removed and in many cases the apex fails to regenerate after the removal of 60μ .

Measurements were made on longitudinal sections to determine how many cell layers were removed when the apex was decapitated. It was found that a piece 40 to 50μ in length included three cell layers. Below this the arrangement of the cells was less regular and measurements could not be made. It was inferred that the smallest piece that was removed was three cell layers thick.

When regeneration occurred after the removal of 60 to 80μ the new apex appeared as a very small hump as in Nos. 226 (Pl. I, phot. 3) and 273 (Fig. 20) and gradually attained normal dimensions. When a smaller piece was removed the new apex was considerably larger from the first. In four plants which were examined after 2 to 3 weeks the new growing point was so small that it seemed probable that its growth had been arrested as in No. 226 (phot. 3). This subject was not investigated further as there was good evidence of the continued growth of the apex in other cases. It was found that the outgrowth of axillary buds did not prevent regeneration from taking place.

Altogether 50 plants of *Vicia faba* regenerated after decapitation.

REGENERATION AFTER OTHER OPERATIONS

The method of injuring the apex by a prick was a comparatively easy operation. The prick was made as median as possible. Regeneration followed in nearly every case, in the same manner as after decapitation or splitting. Twelve positive results were recorded for *Vicia faba*.

When regeneration occurred after a decapitation or prick more than one new apex was never produced; whereas Linsbauer described two apices in *Phaseolus* and *Polygonatum* and a circular growing point in *Helianthus*. As it was not evident why only a single point of the original apex of *Vicia faba* should be capable of continuing the growth a fourth type of operation was performed, in which decapitation was followed by a split. Three of the plants examined after this operation were found to have produced two apices, one on each side of the split, as in No. 235 (see Fig. 7). In six plants a single apex grew out and in six no regeneration took place at all. It was considered that these three results were sufficient to prove the possibility of the formation of two apices when the decapitated stump was split.

CONCLUSION

It is concluded that regeneration takes place in the manner described by Linsbauer, the new apex growing out from the undamaged surface of the original growing point. It was never differentiated internally in the manner described by Reuber for *Populus nigra*. In this respect the stem apex differs from that of the root in which the new meristem is always differentiated internally. Moreover the apical meristem of the root is intercalary, lying within the root cap, while the stem growing point includes the superficial tissues. It has been shown above that the removal of three cell layers suffices to cause the formation of a new growing point.

Whether or not regeneration takes place after an operation depends on the presence of a sufficiently large surface area of the original apex either above or between the youngest lateral members. This condition is fulfilled most easily after a split or a prick, less frequently after a decapitation. It was not found possible to tell which of the cell layers of the original meristem gave rise to the new growing point.

The question remains why the apex of the Lupin inflorescence failed to regenerate. It is suggested that the apex in this state is not capable of regeneration, being too near the end of its growth. A similar failure of an inflorescence to regenerate was described by Linsbauer for the sunflower head. He states that regeneration only occurs if the operation is performed at an early stage in the development of the capitulum. It is uncertain whether the apex of *Vicia faba* also lost the power of regeneration at a later stage; many negative results were obtained after the decapitation of plants which were over 3 weeks old, but this may have been due to the difficulty of operating upon an apex which was turned to one side.

SUMMARY

1. Regeneration after decapitation, a median split or a prick has been shown to occur in *Vicia faba* and *Lupinus albus*.
2. Only that part of the growing point which lies above or between the youngest leaves is capable of giving rise to a new apex, as stated by Linsbauer. Consequently regeneration depends on the presence of a sufficiently large surface area of the original growing point between the wound and the youngest lateral members.
3. In no case did regeneration of the apex take place from the wound surface.



Phot. 1. *Vicia faba*. Johnson's Long Pod. The normal apex in the plane of the stipules.
 $\times 100$.



Phot. 2. *Vicia faba*, No. 178. Seven days after a decapitation. 40 to 50 μ was removed.
 The wound is visible on the left. $\times 100$.



Phot. 3. *Vicia faba*, No. 226. Three weeks after a decapitation. 60 to 70 μ was removed.
 The wound is seen to the right of the new apex. $\times 100$.

PILKINGTON—REGENERATION OF STEM APEX

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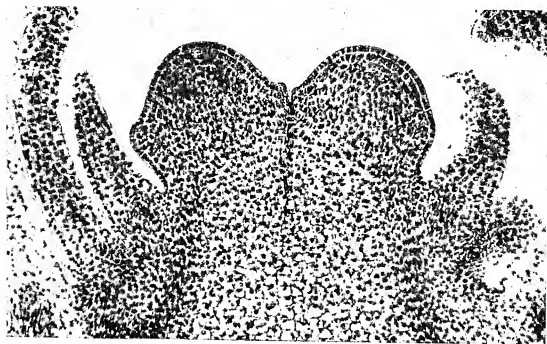
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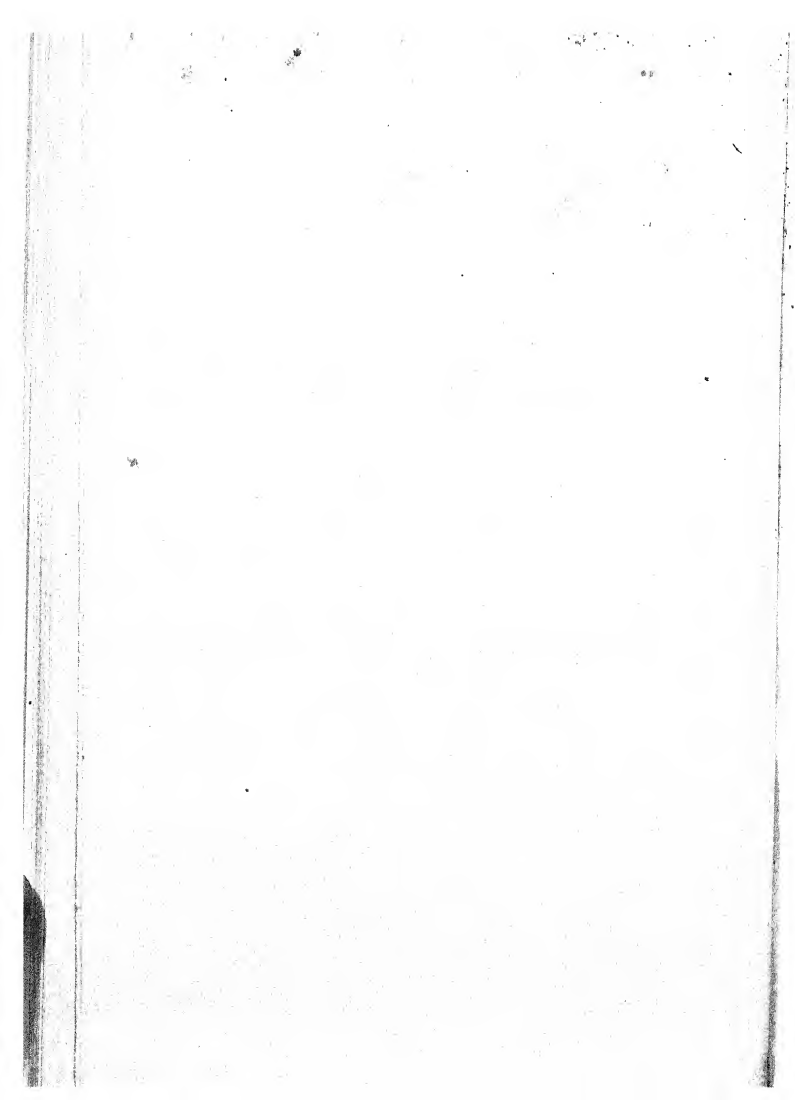


Phot. 4. *Lupinus albus*, No. 73. Two months after being split.



Phot. 5. *Vicia faba*, No. 170. Seven days after being split. $\times 100$.

PILKINGTON—REGENERATION OF STEM APEX



4. Regeneration in *Vicia faba* generally occurred if the length of the piece removed measured from 40 to 60 μ , less frequently if it lay between 60 and 80 μ and never if the piece exceeded 80 μ in length.

5. Two new growing points are only formed when they are separated by a longitudinal split.

6. The continued growth of the new growing points of *Lupinus albus* was followed for several weeks and found to be entirely normal. The wound meristem and formation of new vascular tissues in this region are described.

I should like to express my best thanks to Mr R. Snow, who suggested this investigation, for advice and encouragement during the course of the work and particularly for criticising the manuscript. I am indebted to my father for the photomicrographs.

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THE DEPARTMENT OF BOTANY
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CELL GROWTH AND CELL DIVISION IN THE SHOOT OF THE FLOWERING PLANT¹

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(With Plate III and 3 figures in the text)

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INTRODUCTION

THE problems of cell physiology discussed in the following pages are very difficult and by no means ripe for solution. They are, however, an essential part of the full set of problems presented by the causal anatomy of the vascular plant and it is hoped to show that a discussion, even in the tentative manner that alone is possible at present, by bringing into prominence considerations often neglected or unrecognised, is of material assistance in the consideration of the growth and organisation of the Angiospermous shoot.

Shoot organisation is, of course, determined in the growing point and this discussion can be confined to the meristematic apex and the first few nodes and internodes below. In discussing cell activity in this region it is necessary to contrast three different types; not that it is intended to suggest that the transition from one type to the other is sudden and complete, but these types of cell activity can be readily distinguished and can be seen to be differently affected by the immediate environment of the cell. The different types which must thus be discussed are (1) the meristematic cell, (2) the vacuolating dividing cell, and (3) the vacuolated extending cell. These categories are imperfectly defined by these brief phrases; the meri-

¹ The substance of this paper was communicated in September 1928 at the British Association Meeting in Glasgow to a joint meeting of Sections D, I and K which was organised by the Society for Experimental Biology.

stematic cell has other characteristic properties than that of repeated cell division; the vacuolating dividing cell is also extending. The phrases are used for provisional definition and these different types of cell activity will now be more fully examined and contrasted. Their relation to their immediate environment will then be discussed, because when a large mass of actively growing cells remain in close contact, as in the Angiosperm shoot, the immediate surroundings of the individual cell become part of the machinery of internal correlation of growth.

THE MERISTEMATIC CELL

The writer has tried on different occasions (21, 23, 25) to define the characteristic features of this type of cell. It is relatively easy to recognise in practice, but difficult to define unexceptionably. In properly fixed material, after microtoming and staining, it is readily recognised as a small cell with dense, protoplasmic contents and prominent nucleus. The round nucleus is of normal size, but in such a small cell it bulks large in proportion to the rest of the protoplasm and thus appears in practically every section of the cell. No vacuole is recognisable, and the protoplast should not be retracted from the wall. Furthermore, this wall, which is very thin, often stains with protoplasmic stains, though this is more marked at the root apex than at the shoot apex. The wall is thus relatively inconspicuous, especially as in a tissue of such cells *no intercellular spaces are present*. The cells are singularly free from characteristic inclusions; chondriosomes may be demonstrated by special technique, but normal plastids are minute or invisible and no special granular food reserves are present. Maceration shows the cells to be roughly 12- or 14-sided figures, with the walls of adjacent cells meeting each other at an angle of about 120° .

It is now necessary to consider what these observations from the dead tissue can tell us as to the living cell.

Primarily this cell is clearly composed of protoplasm; nucleus and cytoplasm are all plasm and the wall is often impregnated with it, to judge from its behaviour to stains. Living protoplasm is usually, probably, a fluid aggregate, within which a drop of water, if present, would tend to assume a spherical form. In this meristem cell visible drops of water are not present, but one fluid drop of plasm, the nucleus, is rounded off within the surrounding fluid mass of cytoplasm, with which it remains immiscible under normal conditions (Lepeschkin (17)). A primary characteristic of this fluid mass would

appear to be its plastic nature. If free in water it would undoubtedly assume a spherical form, and if it had any power of resisting the deforming forces resulting from the pressures of its neighbours it would still tend to round itself off from them. But the manner in which it makes close and continuous contact with these neighbours is clear and emphatic evidence that it yields readily under pressure, so that a group of such cells forms a continuous protoplasmic mass, quite free from intercellular spaces. The result is that the space occupied by the meristem approximates to a space homogeneously partitioned into symmetrical equal sized cells. If each cell was in contact only with six of its neighbours, such a homogeneous partitioning of space would result in cubical cells, and the microtome examination of a meristem often suggests that they are roughly cubical in form. But each cell can easily be seen to be in contact with more than six neighbours and maceration shows that the cells approximate more nearly to the shape of the dodekahedron or tetrakaidekahedron, the symmetrical 12- or 14-sided figures by which space can also be homogeneously partitioned (D'Arcy Thompson⁽⁴⁴⁾ pp. 336-8).

It is this characteristic plasticity which sharply marks off the meristematic from the vacuolated cell. In both cells the protoplasm, though fluid, is not homogeneous, and includes in its composition an aqueous liquid, which, if the disperse phase, is in ultra-microscopic drops. In the meristematic cell there is little or no tendency for these drops to increase in size as they do in cells at a later stage of development, when they fuse and grow into the visible vacuole, owing to the presence of osmotically active substances in the aqueous liquid. In the vacuolated cell, therefore, there is an internal force at work, tending to expand the cell, to round it off from its neighbours and to withdraw water from them. Certainly such vacuolated cells may thus withdraw any excess water from meristematic neighbours since these latter seem to have a minimal tendency to expand by taking up additional water, although water must, of course, be firmly held in the highly hydrated colloidal protoplasm. In all probability this tendency not to take up additional water, but rather to part with it to neighbouring vacuolated cells, is a valuable property of the meristematic cell. Pearsall and Priestley have emphasised⁽¹⁹⁾ the fact that such a tendency will facilitate the progress of chemical changes of the general type $A + B \rightleftharpoons C + H_2O$, in the direction of the formation of complex substances C from simpler ones A and B , with the elimination of water. Such chemical changes,

of the general nature of condensations, are characteristic of synthetic metabolism, and it seems probable that in such cells all the protoplasm, both nucleus and cytoplasm, is actively engaged in the work of constructing protoplasm. Each meristematic cell is, therefore, as a result, steadily increasing in mass and pressing against its neighbours. The plastic wall offers little resistance to this slow expansion so that each cell remains in continuous contact with its neighbours over all its surface. When its mass has increased to a certain point cell division follows, and the narrow range of cell size usually visible in the apical meristem suggests that division follows promptly upon the attainment of a certain upward limit of mass.

From the nature of the activities of the meristem cell a close connection may be anticipated between cell division and cell size. All the protoplasm of the cell is engaged in protoplasmic synthesis and the necessary supplies of food material for this synthesis must all enter through the surface of the cell. When this is at its minimal area the ratio of surface to mass is greatest, food supplies are most adequate and synthesis may be expected to proceed most rapidly. As increase in cell size occurs, the proportion of surface to mass falls off, the supply of food to the protoplasm through its surface is less adequate and a fall in the rate of growth may be anticipated. As the rate of growth slows up, at a certain stage the nucleus divides and then, in a region of the cytoplasm farthest removed from the two nuclei, the cytoplasm ceases to form protoplasm but forms carbohydrate instead and a new wall appears. But immediately this new wall forms, a medium is provided through which solutes may diffuse to the surface of the daughter protoplasts, the proportion of surface to mass is thus again increased and synthesis proceeds with renewed vigour. From this standpoint it is clear that the *walls* are the channels of transport for the food supplies to the meristematic cell. *Food supplies could not reach a meristematic cell across a neighbour with an equal need for the same substances to maintain its own activity.* There seems no escape from this conclusion which, so far as I know, is nowhere expressed in the literature of the meristem and which is of great theoretical importance. As is pointed out elsewhere⁽²³⁾, these considerations throw considerable light upon the relatively slow growth of the relatively large meristematic apical cells of many vascular cryptogams, which grow and divide more slowly than the smaller meristematic protoplasts cut off from them. They are also of considerable interest in relation to the predominance

of multicellular over coenocytic types of plant organisation ((23), (30) pp. 71-79).

When the apex of the growing shoot is examined in microtome preparations, it is remarkable how characteristic and constant the pattern of the meristematic tissue is in any one species. Within a certain range, which only the limitations of language seem to render difficult of definition, a series of ordered patterns appears to follow regularly in the development of any shoot apex (Schüepp (31, 34, 35), Schmidt (29)), and such constancy of symmetry in the behaviour of a plastic, fluid mass suggests that each cell division must take place in accordance with definable laws. If these could be correctly enunciated, presumably the ultimate shape and form of the shoot, resulting from cell divisions obeying these laws, should be capable of prediction. Unfortunately, there is considerable doubt and discussion as to the laws governing the divisions in the meristematic mass.

There seems little doubt about the first general law. The narrow limits of cell size met with in the apical meristem, the uniformity of structure in the individual cell, and observations upon the process of division, all suggest that each cell division approximately corresponds to the rule (the first of the laws laid down by Sachs (28), p. 434) that the living cell, free from inclusions of inert matter, divides into two equal masses. The nucleus divides first, and the complex process of karyokinesis determining the equal partition of nuclear matter between the daughter nuclei suggests that *internal* forces are active in determining division, so that when the question of the plane of division arises, it is natural to assume, as Hofmeister (13) did, that the internal force determining the direction of growth in the cell decides the plane of division, which occurs at right angles to the direction of cell growth.

It is very difficult, however, to assess correctly the factors governing the position of the new cleavage plane in the growing protoplasmic mass. Any such new wall may be regarded as a new surface in a fluid medium and, therefore, will tend to obey the laws governing such films at the surface of a liquid. If the fluid protoplast were free to move in water it would assume a spherical form, obeying the law that in any such liquid mass the surface tends to be minimal in area. Similarly within the geometrical figure of the liquid protoplast, adopted under external pressure, the new surface would tend to be minimal in area and, therefore, at right angles to the long axis, if the cell is more extended in one direction than another. This law,

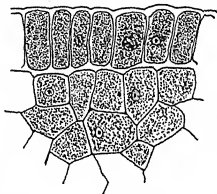
clearly enunciated by Errera⁽⁵⁾ and discussed by Berthold⁽²⁾, seems to be frequently, though not universally, obeyed by the dividing meristematic cell. Hofmeister's law would assign the same position to the new wall in most cases, but while Hofmeister's law may generalise the results of observation it does not indicate an underlying physical principle. Errera's law seems, therefore, the natural working hypothesis to adopt in interpreting the distribution of the new walls in the segmenting meristem. It permits in some cases of an elucidation of the contrasted 'behaviour of surface layers of meristem, stretched tangentially to the surface by the vacuolating tissues below, in which the new walls are regularly anticlinal, and the more deeply situated meristematic cells more regular in shape under more uniform tissue strains, in which the new division walls follow one another in different planes at right angles⁽²³⁾.

At present we cannot know whether such a simple principle as surface tension can safely be applied to explain the position of the new wall. This certainly appears first of all simply as a surface of partition between two protoplasts, which are probably fluid, but, as Giesenhagen points out⁽⁸⁾, its position may alter before it becomes a wall of complex structure and relatively solid properties as a result of the accumulation of substance at the new surface. Giesenhagen has suggested⁽⁹⁾ that it should be regarded rather as the plane of partition between two immiscible liquid drops, so that its final position is the result of the natural grouping of such drops, each tending to assume as nearly as possible the spherical form. This is only another way of arriving at Errera's generalisation that the new wall will occupy a position of minimal area, and in the case of the meristem is not so appropriate, because the protoplast shows no tendency to round itself off from the wall, which is thoroughly permeated with the protoplasm. The surface of separation, however, is never the homogeneous film of Plateau's experiments and calculations, and protoplasmic contacts continue across it, the sites of future protoplasmic connections. All that can be said is that the behaviour of the more complex system is the same in many cases as it would be if the laws of surface tension were obeyed. Furthermore apparent exceptions are sometimes very suggestive if studied from this standpoint.

Thus an apparent exception is sometimes provided by the dermatogen, the superficial layer of the shoot. With great regularity, the walls of this layer are anticlinal. Sometimes the shape of the protoplasts, extended parallel to the surface, might provide an

explanation, but there are many cases (Text-fig. 1) where, although the cells themselves are extended with their long axis at right angles to the surface, all division walls continue to form anticlinally. This apparent exception has been dealt with by De Wildeman (49) and D'Arcy Thompson (44). The outer wall of the dermatogen is in contact with air, and on this surface the wall, throughout its thickness and in contact with the protoplast, is no longer a fluid film. On this side, therefore, as the cell divides the opposed surface tension effects are exerted between the two liquid surfaces of the plane of cleavage, lying against the external solid wall. Under these conditions the new wall forms at right angles to the solid surface.

Sachs's second law stated that each succeeding plane of division tended to occur at right angles to the preceding wall ((28) *loc. cit.* p. 434).



Text-fig. 1. Surface of a developing apple fruit in section. The epidermal cells are still meristematic and divide only by anticlinal walls although they have their long axis at right angles to the surface.

This is a rule with many exceptions and when true, as D'Arcy Thompson points out ((44) p. 348), the new wall usually conforms to Errera's law. But that the new wall approaches the old wall at right angles, even in certain cases of curved or oblique septa, may be because in these cases the older wall represents a solid surface. The most valuable contribution which Sachs made to this problem, as D'Arcy Thompson emphasises ((44) p. 398) is his assertion "that the manner in which the cells divide is the *result*, and not the cause, of the form of the dividing structure."

One point of great interest is any subsequent adjustment of position in the plastic wall. After a new wall has formed, the newly formed cells, subject to the pressure of their neighbours, tend as they grow to assume the original shape of the parent cell before division. Such adjustments in shape are sometimes thought of as

taking place by "sliding growth," in which the wall of one cell is assumed to move over the surface of its neighbours. All protoplasmic connections must be broken in such a process, to be re-established subsequently when relative cell positions are stabilised. This is a hypothetical explanation of the frequently observed changes of cell form, and in the case of the meristem seems innately improbable. The cellulose walls of neighbouring cells are very thin and plastic. They are embedded in a thin slime of mucilaginous pectin and both pectin and cellulose are still penetrated over wide areas by protoplasm so that neighbouring protoplasts are still in continuous connection. It seems much more reasonable that adjustments in shape in such a system should take place by a movement of the whole framework of walls together so that the shapes of neighbouring protoplasts are readjusted under their mutual pressures, without any movement of their individual walls relative to one another.

We have now a picture of the living meristematic cells and of the behaviour of a tissue of such cells at the apical growing point. Plastic, colloidal, almost fluid masses, in close and continuous contact with one another, they adjust their shapes continually under their mutual pressures and tensions and increase in number as they synthesise more protoplasm and divide. The new division walls are apparently governed, in their original position, by the laws of surface tension applying to the distribution of surface films in liquid aggregates.

THE POSITION AND NUTRITION OF THE SHOOT MERISTEM

We have yet to consider the position of the meristematic tissue in the growing apex and the conditions governing its nutrition—conditions which must be of fundamental importance to the life of a tissue which is continually engaged in the work of protoplasmic synthesis.

In the Angiosperm shoot, in contrast to the root, the meristem is always superficial in position. In such a tissue, which is characterised by one activity—its continued increase in protoplasm and consequent cell division—the frequency of cell division becomes an index to its activity, and as each cell division takes approximately the same duration of time, the percentage of cells in any layer found in certain readily recognisable stages of cell division gives an indication of the degree of meristematic activity characteristic of that layer. Applying this criterion, Schüpp (30, 36) finds in the shoot apex

of *Lathyrus* that all layers of the meristem proper, from dermatogen to plerome in classical but now really obsolete⁽²³⁾ terminology, are equally active in division. These active meristem cells clothe the whole surface of the shoot apex. The layers of meristem cells are never very numerous, but there are more in some plants, such as *Hippuris* and *Eloëa*, than in others, such as *Syringa* (Pl. III) or *Scrophularia* (Schmidt⁽²⁰⁾), though in his preparations the removal of cell contents makes the determination of the limits of the meristem less certain). In the same species the depth of meristem differs at different times or under different conditions. Thus at the time of flower formation, the meristematic layers at the apex lie more thickly and they also increase in number during growth in darkness⁽²²⁾, when the activity of the superficial layers is greatly diminished.

As has been discussed elsewhere⁽²³⁾, this superficial position of the meristem, coupled with the fact that the outer layers, the dermatogen and periblem of Hanstein⁽¹⁰⁾, the phyllogen of Schwarz⁽³⁷⁾, whilst growing as fast as the layers within, multiply entirely by walls at right angles to the surface, goes far to explain the exogenous production of leaf and bud primordia, although the emergence of such a primordium is always associated with the multiplication of the cell layers in the young primordium by the appearance of periclinal walls in the sub-epidermal layers.

It is necessary now to consider how the superficial meristem is supplied with food. The ultimate source of this food supply is undoubtedly the vascular system, but this terminates some distance below the position of the meristem. In the shoot apex sometimes no lignified xylem element can be detected within one or more millimetres of the apex. The differentiated xylem element is undoubtedly completely permeable so that the liquid it contains, when under pressure, will flow from it into the walls of the neighbouring cells. In the young and turgid shoot apex, these walls are normally saturated with water from vascular system to superficial meristem, and there is no indication of any impediment between vascular supply and meristem which would prevent movement of water or water-soluble solutes along this continuous path, composed mainly of a series of carbohydrate gels⁽⁴⁵⁾.

Water and solutes *may* also move across the protoplasts, but we know on good experimental grounds that the vacuolated protoplasts of the intervening region absorb and retain water osmotically and allow solutes to enter them much more readily than they allow them to leave (Steward⁽⁴¹⁾). There seems, therefore, to be every reason for

regarding the *walls* of the intervening tissue as the natural path by which such movement takes place. We have already seen that in the meristem itself, in which the walls are very thin, the solutes are probably entering the protoplasts all over their surface, and therefore are moving along the intervening walls. If the solutes are withdrawn so rapidly, to supply the needs of the meristematic protoplasts, then the supplies available in these thin intervening walls could not maintain an indefinite depth of meristematic tissue and possibly the relatively narrow depth of this tissue is connected with this fact. In the etiolated plant it is true that the depth of meristem is greater, but the outer layers have practically ceased to grow: in the apex of the flower bud there is a greater depth of active meristem, but there is also some suggestion in the appearance of the apex that the intervening walls are thicker, a fact possibly correlated with the relatively greater proportion of carbohydrates in the supplies to the apex at the period of flower formation.

Very little water, if any, is absorbed by the meristematic protoplast whilst active in synthetic metabolism, but solutes must enter very readily, and this, in view of the normal semi-permeability of protoplasm and the experimentally established slow rate of penetration of inorganic solutes into the vacuolated protoplast, is a phenomenon of considerable interest. Michaelis and his colleagues have investigated the permeability of protein-impregnated membranes, such as the surface of the meristematic protoplast undoubtedly must be. These protoplasts need to absorb readily both anions and cations and Michaelis and Nagoya (18) point out that protein-impregnated membranes prove to possess this property when they are immersed in a fluid which has a reaction close to the iso-electric point of the protein in the membrane. The meristematic protoplast is, therefore, behaving in this respect as if it were bathed in a liquid with a hydrogen-ion concentration close to that of the iso-electric point of the main constituent protein in its surface. This is a suggestive fact, especially as Pearsall and Priestley have already pointed out that it is exactly under these conditions that the protoplast might be expected to exhibit minimum affinity for water, a condition that would favour its chemical activity in synthetic metabolism (19), see also Weber (47)).

If, then, solutes can reach the walls of the meristematic protoplasts from the vascular supply, their absorption can be understood, but their continuous supply from the relatively distant terminus of the vascular supply raises another problem. A. V. Hill has recently pointed

out the significance of distance in the case of delivery of solutes by diffusion through a liquid medium to the living cell (12) and undoubtedly the distances involved at the shoot apex makes it difficult, if not impossible, to assume that movement of solutes along the walls by molecular diffusion could maintain an adequate supply of food. Such an assumption is not necessitated, however, by the existing conditions. Between meristem and vascular supply is a tissue of growing vacuolating cells. Such cells are continually differentiating from the inner surface of the meristem and from then onwards continually taking in water. This water is all drawn from the vascular supply which also has to make good the loss due to evaporation through the cuticle, etc. The result is a constant flow forward of water from the vascular supply along the walls of the tissue intervening between the terminus of the lignified elements and the base of the meristem. These walls, mainly carbohydrate in composition, are sufficiently porous to permit of such a flow of liquid in mass along them and with this liquid the necessary solutes will be carried forward, far faster than by diffusion over the distances concerned. Cell differentiation is a process that must proceed sporadically, cell by cell, so that the demand for water will not be uniform; similarly the pressure of sap in the vascular supply will vary throughout the twenty-four hours, with alternation of day and night and with the phases of vascular differentiation (see p. 75). The result is that an ebb and flow may be anticipated in the mass movement of water along the walls of this intervening tissue. Such an ebb and flow will assign a still more dominant place to mass movement of liquid rather than diffusion in the transference of solutes from the vascular element to the relatively distant meristem.

It thus seems possible to visualise the conditions under which the superficial meristem of the shoot apex may be maintained in food supplies during its growth activity. In this process the carbohydrate nature of the walls intervening between meristem and vascular supply plays a predominant part (21, 23); along these walls a flow of nutrient sap takes place under conditions which may be more fully realised later, when the activity of the tissues below the meristem have been passed in review. We must now pass to a consideration of these tissues, lying beneath the superficial meristem, which consists of vacuolating cells which still continue to grow and divide.

THE VACUOLATING AND DIVIDING CELL

The writer has found the consideration of this type of cell growth in a separate category of very great help in the elucidation of the apical organisation of the shoot. Up till now this distinction does not appear to have been clearly made. All dividing cells have been lumped together into the meristem and as a result, as will appear below, some of the definitions employed by Schüepp⁽³⁸⁾ in his monograph upon the meristem really apply to tissues composed of cells of this general character. And yet, although one of the two cell types develops from the other gradually at the growing apex, they are, when fully developed, very differently characterised. The metabolic tendency that marks the transition appears to be a tendency to substitute, in part, for protein synthesis, a synthesis of carbohydrate. As a result, the zone of transition from one type of tissue to the other is often indicated by a region of still unvacuolated cells which contain starch. This zone can readily be recognised in the etiolated shoot; in the light these cells vacuolate so rapidly, with hydrolysis of the starch, that their presence is not so easily established⁽²²⁾. Their detection is hindered by the fact that this starch is often so intimately combined with protein that no reaction is seen with iodine reagents until after previous treatment with such an agent as Eau de Javelle. These cells have, therefore, been described as containing "masked" starch, and seem to occur very frequently in the shoot of Dicotyledons (Swarbrick⁽⁴²⁾). In some cases, as in the apex of the stolon of the potato during tuberisation, the formation of this type of cell seems to be long maintained.

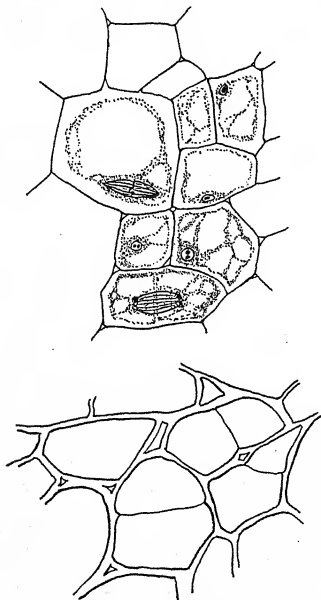
Cellulose is also formed more freely at the surface of the protoplast at this stage, and very soon visible vacuoles appear, probably as the result of the release of soluble carbohydrates into the watery disperse phase of the cytoplasm. At the same time the cell wall becomes more elastic and offers more resistance to the outward pressure from the vacuole, so that the cell tends to round itself off from its neighbours like a tightly blown up ball. These forces come into play at a time when the cells are cemented together by a plastic pectin matrix. This yields and the cells pull apart from one another, rounding off the originally angular corners where the cells were kept in contact by their mutual pressure, and at these points intercellular spaces now arise. It is impossible to identify these spaces with Plateau's *bourrelet* or "surface of continuity," or to regard them as drawn from the material of which the partition wall itself is com-

posed as D'Arcy Thompson alternatively suggests ((44) p. 298). The intercellular spaces are at first full of liquid, the same sap which is moving in the walls in the manner discussed in the previous section. Harting describes how in *Phytolacca*(11), at an early stage in shoot growth, the rapid multiplication of cells in the periphery, as the result of repeated divisions by longitudinal radial walls, followed by cell extension, causes the peripheral tissues to move outwards rapidly, so that they pull upon and tear apart the cells at the centre of the stem, creating a pith cavity which at first is still full of sap.

Cellulose now forms more rapidly at the protoplast surface so that in spite of cell extension the wall grows thicker. New cell walls can, therefore, be distinguished from older ones by their difference in thickness. The intercellular spaces are also wider at the angles between the older cell walls. These two characteristics permit the recognition of groups of cells which have arisen from a common parent. In the meristem proper this recognition is difficult, if not impossible; there are no intercellular spaces and all cell walls appear about the same thickness. Schüepp's distinction(36) between "Rippen," "Platten" and "massige" meristem, therefore, applies rather to tissues composed of vacuolating, dividing cells than to "meristem" in the narrower sense. In the shoot apex most divisions in these cells take place by a series of transverse walls so that the group of cells formed from the original cell are in a short filament, the "Rippenmeristem" (Pl. III). Divisions occur in two planes at right angles in the mesophyll of the developing leaf, thus giving the "Plattenmeristem"(33). In the flesh of the apple an excellent example is seen of divisions in three planes at right angles giving the "massigemeristem" (Text-fig. 2).

The factors which determine cell form and the plane of cell division in the non-vacuolated plastic cell need not be operative in the same way in the very different conditions governing cell extension and growth in the turgid vacuolated cell. In the first place such cells are pressing upon one another like balloons inflated to the point of rigidity. They may, therefore, move relatively to one another by a type of true "sliding growth." Certainly the appearance of the rows of "Rippenmeristem" in the young internode of the stem suggests that the end cells of such short rows are inserting themselves between their neighbours (Pl. III). Such movement will usually be slight in amount and will stop when, as described later, the plastic matrix of the middle lamella sets to a hard, dried mass of insoluble soaps and pectates.

The factors which govern the position of successive division walls and so determine the adoption of the "Rippen," "Platten" or "massige" type of tissue are very difficult to diagnose. Cell division



Text-fig. 2. Groups of cells seen in sections of the flesh of the apple fruit (Wagener). In one figure dividing nuclei are seen in vacuolating cells, in a young stage of fruit development. In an older stage, shown without cell contents, the younger cell walls are readily distinguished by their small thickness and by their connection with smaller intercellular spaces.

in vacuolated cells seems to be initiated by the nucleus, and cases have been reported where the new cell wall appears first on the side of the cell nearest the asymmetrically placed nucleus and then gradually extends completely across the cell. Zimmermann cited

such cases as arguments against the generalisation of Errera that the position of the wall was determined as that of a weightless film in a liquid medium⁽⁵³⁾. They are rather evidence, however, that the conditions governing this type of cell division are different from those applying in the normal meristem where the wall appears simultaneously across a dense non-vacuolated protoplast. In the vacuolated type of cell Giesenhagen's generalisation⁽⁸⁾ appears to hold, viz. that the position of the nuclear division figure determines the position of the wall, which appears at right angles to the nuclear spindle. But the position of the dividing nucleus in the protoplast often seems to be determined by the general shape of the dividing cell, so that the factors governing cell extension may again be responsible for the position of the new cell wall. These factors are at present very obscure. Since the suggestive papers of Sponsler and Dore⁽⁴⁰⁾ it seems certain that one important factor that may have to be taken into account is the polar structure of the cellulose molecules, which are shown by X-ray analysis to be composed of units linked in regular order in the wall in a pattern which may readily admit of more extension in one direction than another. Not until these problems can be successfully attacked may we hope for light upon the conditions governing the series of cell divisions involved in the production of "Rippen," "Platten" and "massige" types of tissue.

The fact that the cell wall in the vacuolating dividing cell increases in thickness, whilst the protoplasmic mass within remains relatively insignificant in amount, suggests that only a limited amount of the protoplasm present is active in protein synthesis, whilst the rest is engaged in carbohydrate metabolism. Cellulose is formed at the external surface of the cytoplasm; starch and sugar if present accumulate in cytoplasmic inclusions or vacuoles; on the other hand in many fixed preparations the protoplasm seems more densely aggregated around the nucleus. Cell division is also initiated by the nucleus; and all these facts suggest that protoplasmic synthesis may now be centred in the only portion of the protoplasm that remains non-vacuolated—the nucleus. In the case of the vacuolated cells of *Spirogyra*, strong experimental evidence has been brought forward in support of this suggestion. By cooling or by a slight dose of anaesthetic, Gerassimow delayed and disorganised the process of cell division in this plant after it had begun^(6, 7). As a result some cells were obtained with two nuclei or with an abnormally large nucleus, at the expense of others which were without nuclei. If these enucleate cells contain chloroplasts, they may remain alive for a

long time, may form cellulose or starch or extend in volume a little, but they fail to increase in content of protoplasm and never divide. Cells which have an abnormal ratio of nucleus to protoplasm as a result of such experimental treatment gradually return to the normal as the result of subsequent divisions. Thus, if the nucleus is abnormally large, division is delayed till later than usual until a large quantity of cytoplasm has been accumulated, so that in the daughter nuclei the normal nucleus-cytoplasm ratio is approximated. Thus in these vacuolated cells there seems to be a stable nucleus-cytoplasm ratio, which is understandable if the maintenance of the total quantity of protoplasm in this type of cell depends upon the activity of the nucleus alone. In one type of meristematic, non-vacuolated cell, the cambium initial, this nucleus-cytoplasm ratio is clearly quite disregarded. As is pointed out elsewhere⁽²³⁾, if in this type of cell all the protoplasm is engaged in protein synthesis, less significance attaches to the nucleus-cytoplasm ratio than to the proportion between surface and mass.

THE POSITION AND NUTRITION OF THE VACUOLATED DIVIDING CELL

The type of vacuolating dividing cell with which we have been dealing in the last section is found for a considerable distance below the superficial meristem and is characteristic of that region of cell growth and activity which lays down the node and internode of the "articulate" shoot organisation. In the node, near the leaf insertion, a region can usually be distinguished where but few divisions have taken place and these not all in the transverse plane. The elongation of the internode on the other hand is clearly due to the multiplication by a series of transverse divisions of a growing number of parenchymatous, dividing vacuolated cells. Furthermore, the comparative lengths of a series of internodes upon the same shoot have been shown by Moll and Tammes⁽⁴³⁾ to be attributable to the number of cells thus formed, not to the relative length of the individual cells. It is this process of repeated transverse division, taking place throughout the internode, which is responsible for an early period of uniform growth throughout the internode, described particularly by Harting⁽¹¹⁾ and Wiesner⁽⁴⁸⁾. Usually, at any moment, only the two or three uppermost internodes, just separating from the apex, are in this stage of uniform growth throughout their length.

The vacuolated cell does not synthesise protoplasm as fast as a cell of the superficial meristem; its extension in size may be more rapid, but this is due mainly to the intake of water into the vacuole

and into the hydrated protoplasm. Cell divisions also are not so frequent in occurrence in this tissue, but they are numerous and long maintained. A large amount of carbohydrate is also deposited upon the extending walls of these cells, so that their activity requires a constant supply of food. The source of supply is again undoubtedly the vascular strand. If the nutrient solutes have once entered a vacuolated cell, in view of our knowledge of their relative impermeability when leaching in water (Steward⁽⁴¹⁾), it is doubtful if they will be released again, so that the supply for the more distant cells probably reaches them along the walls rather than across the intervening protoplasts. So long as walls, and *intercellular spaces*, are filled with sap, there will be no difficulty in the transfer of solutes by this path to the actively growing cells. The activity of the meristem cell in synthesis seemed to depend upon the maintenance within a very narrow range of pH of this aqueous sap. In the vacuolated cell, if protoplasmic synthesis is restricted to the nucleus, which is buffered against this external sap by the cytoplasm intervening, synthesis can probably continue over a wider range of hydrogen-ion concentration, though the rate of swelling of the protoplasts will be influenced by this factor (Pearsall and Ewing⁽²⁰⁾).

The characteristic activity of the vacuolated dividing cell, like that of many an algal cell, is thus seen to depend upon its maintenance in an aqueous medium. This is an interesting conclusion as to the activity of the type of cell which is present in great numbers beneath the superficial meristem of the Angiosperm shoot, which represents the most specialised development of plant organisation upon land. It is an interesting problem, which must be postponed for the moment, how the necessary aqueous environment is maintained for these cells in the subaerial tissues of a land plant. That this condition is necessary for the proper functioning of this type of cell is emphasised by a consideration of the third category of cell contributing to the growth of the shoot. This type of cell, vacuolated and much extended, but no longer dividing, appears when the intercellular spaces in the tissues of the shoot are no longer filled with sap.

THE VACUOLATED EXTENDING CELL

In the young internode, first of all in certain regions near its base, vacuolated cells will be found to extend very rapidly in volume, whilst at the same time they cease to divide. This type of cell growth follows comparatively suddenly upon the previous type of slow extension in volume with continued division. It appears also to be

closely correlated in time with the general displacement of sap in the intercellular spaces in this same region, by air moving up from the older parts of the plant below. With the entrance of air there follows the occasional connection of these air spaces with the exterior through the differentiation of stomata. It is an open question, however, whether these pores are the result or the cause of the extension in volume which follows upon the displacement of sap by air in the internal intercellular spaces. The pores appear in the epidermis at the time that cell extensions are actively proceeding in the outermost layers of the cortex and epidermis. In the shoot the cells begin to vacuolate in the pith, and, very shortly afterwards, in the middle region of the cortex. Vacuolation in the sub-epidermal layers is usually later in developing. It must be left for further investigation to decide whether the stomatal pores, when originally formed, contain air or sap. Probably the air first reaches the intercellular spaces in the developing pith and inner cortex from the internal atmosphere in the older tissues, not directly through new epidermal pores at the same level, these coming later in development.

The connection of air in the intercellular spaces with the rapid increase of the cells in volume, is in all probability causal. In the vacuolated cell, extension depends upon the balance between the expanding forces due to the intake of water by osmosis, and the resistance of the elastic cell wall to this tendency to extension. Ursprung and Blum(46) have shown that in the root apex the osmotic pressure of the cell sap is highest in the vacuolated cells nearest the apex and that as the cells increase in size behind the apex the concentration of the sap falls. On the other hand the suction pressure of the cells increases rather suddenly in a region some distance behind the apex, a fact which can only be attributed to a relatively sudden fall in the resistance of the wall to stretching. This fall in elastic resistance of the wall probably coincides with the appearance of air in the intercellular spaces of the cortex of the root. Ziegenspeck(50, 51, 52) has shown, particularly for the root hair, but also for other tissues in this region of the root, that at this stage the cell walls may show a different micro-chemical behaviour. Protein substances that mask the reaction may have to be removed first by treatment with a reagent like Eau de Javelle, then the wall will react directly with iodine reagents without previous treatment with hydrolysing agents such as concentrated zinc chloride. Ziegenspeck terms this the "amyloid" reaction, and regards the amyloid stage as a transient, highly hydrated stage of the cellulose in which it is

very plastic, but not elastic. He compares it with the parchment stage which can be induced in cellulose fabrics by the use of suitable solutions of sulphuric acid and which when first formed is similarly plastic.

In the root this plastic amyloid stage of the wall can frequently be detected by a suitable manipulation with reagents, it is readily demonstrated at the apex of the extending root hair and can also be detected in other tissues (51, 38). It is always a transient stage and in the subaerial shoot seems much harder to detect. Its association with air is indicated in the readiness with which root hairs appear in moist air, whilst with many roots their formation is repressed by growth in water. Janse (14) has also pointed out that in many roots, injection of the intercellular spaces in the growing region with sap represses the normal cell elongation. In the shoot, the rapid increase in volume of the vacuolated cells at about the time that air replaces the sap in the intercellular spaces around them, seems an indication of the rapid development of such an "amyloid" condition, even if it cannot be detected microchemically because passed through too rapidly.

This argument must not be pressed too far. The displacement of air in the intercellular spaces in the parenchymatous tissues of the shoot appears to synchronise with a rapid increase in cell volume, which may be explained by the development of a plastic "amyloid" stage in the walls of the cells as the result of the presence of the air. But it must not be forgotten (1) that the elongated parenchymatous cells of the stele of the root have never been in contact with air in the intercellular spaces, and (2) that the walls of the phloem, a tissue which is often remote from intercellular spaces containing air, often show the amyloid stage particularly well, without any previous treatment with Eau de Javelle. Similarly, in some roots, root hairs form readily under water.

The rapid increase in cell volume is naturally accompanied by a large movement of water from the intercellular spaces into the cell. At the same time, the spaces are probably increasing in volume as the result of the general expansion of the tissue. The result is that this first entry of air into the spaces is followed by rapid expansion of the spaces and an acceleration of the displacement of the remaining sap by air. This rapid increase in the volume of the cell is quite independent of any increase in protoplasm. After it has taken place the delivery of solutes from the vascular supply along the partially dried walls must very much diminish, and cell growth, as measured by increase in protoplasm and cell division, must come to a standstill. Only with a subsequent injection of the intercellular

spaces with sap, once more displacing the air and bringing solutes freely to the surface of the protoplast, will protoplasmic increase and cell division be renewed. Cases of this kind have been described; they are especially frequent as the result of wounding (26, 27).

In the stem internode it is a very characteristic feature of this period of cell extension that the originally almost isodiametric cell extends mainly in a direction parallel to the long axis of the plant. This may be due in part to the resistance to radial extension offered by the epidermis and cuticle and the stele. A further contributing cause may be the orientation and linkage of the molecules in cellulose. X-ray analysis by Sponsler and Dore (40) show that these molecules are so linked that the entry of oxygen into the molecule can take place more readily at certain points in the linked pattern, with a consequent movement apart of the molecules in one direction in space rather than another. Such movement, associated with the chemical changes in the wall during the production of the "amyloid" stage, might thus determine the direction of cell extension. Sponsler and Dore have analysed an X-ray pattern from adult cells in which diffraction patterns will be due to the secondary lamellae of the wall. This extension in the young vacuolated cell is the result of the air meeting the *primary* wall. The same contact of air with the primary layer of the wall of the xylem element, which always remains un-lignified, is responsible for the sudden bulge of this wall into the xylem element in thylosis, which, as Klein clearly showed (15), is produced by the presence of air in the trachea.

After the first rapid expansion of the vacuolated cell, it may still undergo a further slow increase in volume and relative shift in position as water is still taken in and the strains produced by its first rapid expansion in association with its neighbours are gradually adjusted. Such continued movement will not be possible for long, however. With the advent of air into the intercellular spaces, the walls in contact with it are still chemically changing and these changes lead to a loss both of elasticity and plasticity, so that further changes of shape of any kind are resisted. At the same time the substance of the middle lamella is changing to dried insoluble calcium soaps and pectates, etc., and with these changes all possibilities of sliding growth are lost. Sliding growth in the growing tissue is therefore probably a phenomenon of very limited occurrence, and is limited to the period when the cells have passed out of the plastic meristematic state, but have not yet become too rigid owing to the effect of air in the intercellular spaces.

The three stages through which a cell passes whilst it contributes to the growth in length of the shoot have now been passed in review. It will be seen that whether a living cell plays its part as a meristematic unit, or as a vacuolating dividing cell, or makes its last contribution to growth in the form of a final extension in volume, seems to depend upon its immediate environment. If the cell is surrounded by an aqueous medium, over a limited range of pH it may be meristematic, over a wider range it may be vacuolated, but still capable of growth and division, but when the surrounding sap is displaced by air, a chemical change in its wall may permit of a period of rapid extension before it enters into a stable state as part of the permanent tissue of the plant.

Each of these three stages of cell activity plays a very important part in the growth of the shoot, and in the individual cell each stage follows in a regular sequence, determined by the place of the cell in the complex organisation. If our analysis of the factors governing cell behaviour is correct, this must mean that the sap supply to the growing shoot likewise passes through a series of internally regulated changes. The reaction of this sap has been discussed previously (19, 23). It seems to be determined in part by a delicate balance between the relatively acid contents of the xylem and the relatively alkaline contents of the phloem. If the fluid moving from the vascular supply into the walls is not highly buffered it may be materially influenced in reaction by tissue respiration or aeration. This point cannot be discussed further here, but in conclusion it should be pointed out that the mode of vascular differentiation which is characteristic of the shoot has a very important bearing upon the supply of sap to the apical tissues, and therefore is of fundamental importance in its organisation for growth.

VASCULAR DIFFERENTIATION IN THE SHOOT APEX

It seems to be an invariable characteristic of vascular differentiation in the shoot apex that the new xylem element, which appears in the procambial ring just at the base of the new leaf primordium, is at first isolated from all other already differentiated vascular elements in the shoot. In contact with this first proto-xylem element, others now differentiate, some forming upwards into the leaf primordium and some by its side in the extending internode. Below, however, it remains distinct from the vascular system of the older regions of the stem. This gap of parenchymatous tissue, between the newly differentiated foliar strand and the main vascular

supply, is the foliar and branch gap of the vascular axis. Later short tracheids differentiate from this isolated foliar strand down the sides of the branch gap and, in connection with these tracheids, vascular differentiation continues downwards in the procambial ring of the internode below. The writer has followed this process in the young stem of *Vicia faba*; it is the development of what is known as pectination between two foliar traces superimposed in the vascular ring (De Bary⁽¹⁾). In *Vicia faba*, and probably in many other plants as well, during development this pectinating, downwardly differentiating strand, connected with the leaf-trace which enters two nodes higher up, still remains distinct from the main vascular supply, to which it is not connected at first by any bridge of lignified vascular tissue. The phenomenon thus briefly described is well known, De Bary and all the classic writers in anatomy having emphasised the downward differentiation of the foliar strand, but its physiological significance has not been indicated.

During differentiation a future xylem element passes through a series of phases. At first a meristematic procambial element, it next becomes a vacuolated semi-permeable element, and then more and more permeable, with changes in its walls which, as they thicken, become lignified. Now this isolated, permeable element containing a relatively concentrated sap from its original cell contents, is surrounded by living cells which, in its immediate neighbourhood, are meristematic, thin walled and without intercellular spaces. Through these walls this system is in continuous aqueous contact with the relatively dilute sap in the main vascular supply. An osmotic system, even if an imperfect and transient one, is thus constituted, which will withdraw water from the main vascular supply below and drive it slowly along the intervening walls into the tissue surrounding the isolated foliar strand. That the vascular system of the shoot could function as an imperfect osmotic system was suggested by the earlier experiments of Pitra and Kraus⁽¹⁶⁾ which were later confirmed by Priestley and Armstead⁽²⁴⁾. Every differentiation of such an isolated system thus becomes an agency supplying sap to the meristem above it and the vacuolated dividing cells surrounding it, and so long as this isolated system is in existence the type of cell growth which is characteristic of internode and leaf initial can continue. All this activity of cell growth around such an isolated vascular strand should, therefore, depend upon it and cease when its existence terminates by the completion of a lignified connection from it to the main vascular system. The growing shoot is then organised as a

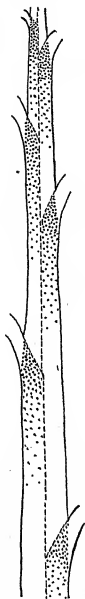
community of tissue aggregates, each grouped around a foliar strand, upon which it relies during development for the supplies to maintain growth. Up till now the shoot has been spoken of as organised in node and internode, but the vascular system does not develop in conformity with this simple structural pattern. Thus in *Vicia faba* the isolated vascular system of a foliar strand extends downwards, unbroken, through two internodes, to terminate above the leaf gap which subtends the next leaf vertically below. The growth activity of the vacuolated cells surrounding this strand is similarly continuous through both internodes and quite unbroken, on the side of the foliar strand, by the intervening node, which really has a structural existence only on the other side of the stem where it is connected with the foliar gap separating two similar vascular systems isolated from one another during development.

THE UNIT OF SHOOT GROWTH

As the characteristics of the later cell growth in the shoot are thus determined by vascular differentiation, and as this proceeds in a series of separate articulated steps, the shoot organisation thus built up is itself an articulated structure. But the growth units of this compound organisation are not node and internode, the "Sprossgliede" of Schüepp(32), or the "Merithalle" of Harting(11), but that segment of the axis which subtends a leaf initial and surrounds its vascular strand as it differentiates, a segment of the axis which runs downwards from a leaf insertion, to the foliar gap above the insertion of the leaf next vertically below. This is the morphological unit of the shoot originally identified by Čelakovský(3) as the "Sprossglied," and identified again on anatomical grounds by Chauveaud(4), who revives a still earlier conception in the term "phyllo-rhize."

When the vacuolated cells of the axis pass over into the third and final growth stage and extend rapidly without any further cell division, this change is primarily associated with the displacement of the sap in the intercellular spaces by air, a process which will depend in part upon the stage of development of the vascular strand. This process of rapid cell extension begins at the base of an internode, but only in the tissues surrounding the vascular strand, which is then in the appropriate stage of development. Thus in *Vicia faba*, this cell extension is proceeding on one side of the axis, whilst on the other side, if the vascular strand is still at a younger stage, cell division with relatively slow cell extension still proceeds. This stage

of cell extension then develops rapidly up the axis on the side around this same vascular strand, passes without interruption into the internode above, and so conforms also to the organisation of the plant in "Sprossglieder" as defined in the previous paragraph. These characteristics of shoot extension explain a puzzling contradiction between Harting's careful measurements of the rate of internode extension and of cell length (11). He measured the extension of different thirds of the internode of *Tilia* separately, and found that at first all three increased in length at about the same rate, but that then the more rapid increase took place at the top of the internode, to be followed by a more rapid increase of the middle third; the base then increased rapidly but extension continued longest at the top of the internode. These results seem quite at variance with his cell measurements which always show that after a stage where all cells are of about the same length the cells increase rapidly in length, but always from below upwards. The contradiction is obviously the result of the unit chosen for examination, which is really, in *Tilia*, a combination of portions of two veritable growth units. The early rapid phase of growth, at the top of the internode, was the result of the rapid extension of the middle portion of the older growth unit; later this was followed by the extension of the younger unit (see Text-fig. 3), the basal portion of this unit (in the internode below) extending first. Apparently extension in the lower median portion of the older growth unit and a certain region of the younger unit synchronised so as to give a temporary maximum rate in the median third of the internode; in any case, extension would cease last at the top of the internode. In his measurements of cell length, Harting would find, irrespective of growth units, that in all longitudinal files of cells in



Text-fig. 3. A diagrammatic representation of a shoot with alternate leaves to indicate the "growth units" composing the axis which are equivalent to the "Sprossglieder" of Čelakovský. Vacuolation occurs later in the upper portion of each unit.

the internode, the long extended cells appear first at the bottom of the file.

It will be clear from the brief account given above of the process of differentiation of this isolated vascular system that the foliar strand in the leaf and the early vascular supply into the young leaf primordium often form one unit of the growth system. It is, therefore, understandable that Moll and Tammes found the development of leaf and internode to be correlated in magnitude(43). In later papers the developmental anatomy of this shoot growth unit will be followed in different types of shoot organisation. A fuller comparison of this growth unit with the morphological unit of Čelakovský and the anatomical unit of Chauveaud will then be possible. It is at least suggestive that a consideration of the physiological conditions governing cell growth and division in the shoot should apparently cause us to define the same unit of shoot organisation as a morphologist and an anatomist each of whom had a different focus of interest.

SUMMARY

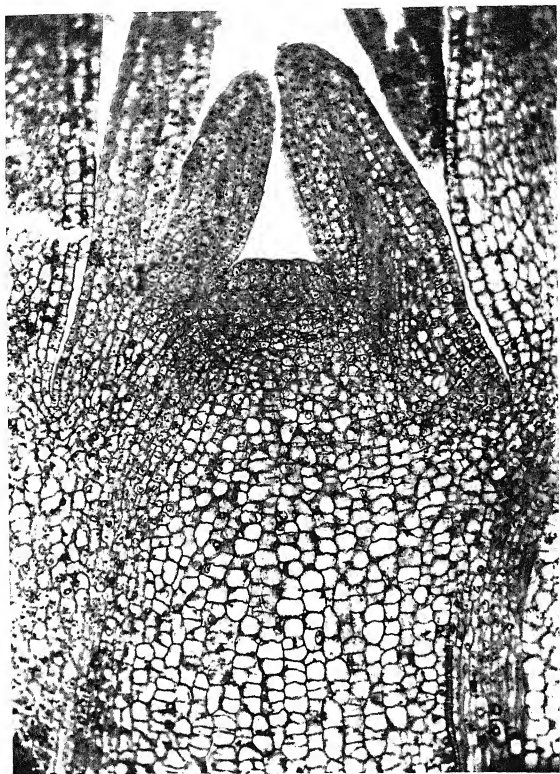
1. The living parenchymatous cells of the shoot apex pass through three phases as they contribute to the growth of the shoot, viz. (1) the meristematic cell, (2) the vacuolating dividing cell, and (3) the vacuolated extended cell which has ceased to divide.

2. The meristematic cell is a fluid aggregate with a plastic wall. Its shape is, therefore, determined by external pressure and the cells lie closely pressed together without intercellular spaces. The position of a new division wall seems to be determined by the shape of the cell and forms in a plane of minimum area, but there are exceptions.

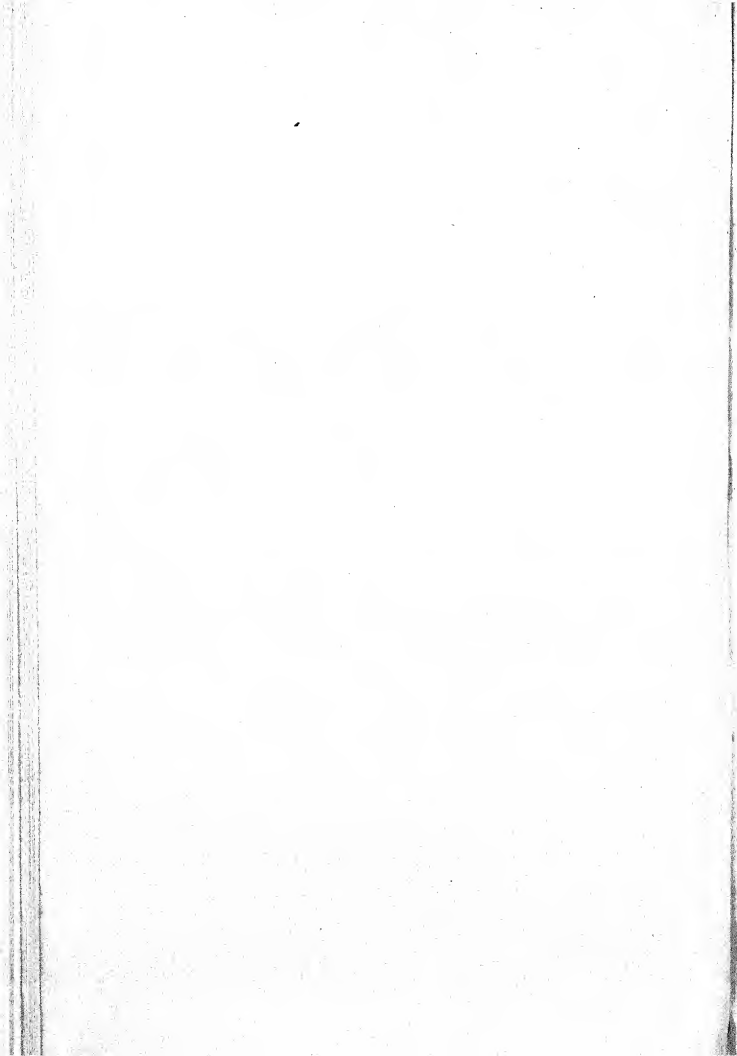
3. The vacuolating dividing cell rounds itself off by an internal hydrostatic pressure directed against an elastic cellulose wall which thickens with time, so that groups of cells can be distinguished as arising from different common parents by the relative thickness of the walls and size of the intercellular spaces. The position of a new division wall seems again to be determined by the shape of the cell, but this will not be the result simply of external pressure as in the case of the meristematic cell.

4. The vacuolated cell may extend greatly in volume at the time that it ceases to manufacture protoplasm and divide.

5. It is suggested that "sliding growth," by the relative movement of the walls of neighbouring cells past one another, is only possible in the stage of the vacuolating dividing cell. At this stage



Photograph of longitudinal section of decussate shoot apex of *Syringa vulgaris* L. $\times 290$. The youngest pair of leaf initials are still nearly completely meristematic, as also are the cells crowning the apex of the shoot. Beneath the apex, the vacuolating but still dividing cells of the pith are visible. In these cells longitudinal extension is associated with frequent transverse divisions ("Rippenmeristem" of Schüpp). On the right, in the leaf initial and at its base, the longitudinally extended, still meristematic, cells of the procambial strand can be seen.



the cells are relatively rigid through turgidity and the intervening middle lamella is still plastic.

6. All three phases of cell activity are discussed in relation to the internal conditions necessary to their maintenance, and it is suggested that (a) the meristematic phase, in which all the protoplasm is engaged in protoplasmic synthesis, is only maintained when the liquid surrounding the cell has a pH near the iso-electric point of the main constituent protein of the cell; (b) the vacuolating dividing cell, in which the nucleus alone synthesises protoplasm, can exist over a wider range of pH , but still requires an aqueous environment; and (c) the vacuolated cell ceases to divide and usually extends greatly in volume when air replaces water in the intercellular spaces.

7. It is suggested that the "amyloid" stage in cell wall development, which can sometimes be detected by micro-chemical reactions, may have significance in connection with the great increase in cell volume, which often accompanies the appearance of air in the intercellular spaces.

8. The maintenance of the sap supply at the cell surface, which is so essential to its activity in either of its dividing phases, is determined by the method of vascular development in the shoot. A series of isolated vascular systems differentiate, in connection with successive foliar initials, each of which in turn acts as a separate osmotic system and withdraws water from the main vascular supply.

9. Each of these isolated vascular systems with the parenchyma surrounding it, comprising a leaf initial and a segment of the axis, behaves as a separate growth unit, of which the characteristic behaviour is determined by the stage of development of the included vascular strand.

10. These growth units are compared with other units, distinguished on various grounds by previous investigators as building up the shoot. They correspond very closely with the "Sprossglieder" of Čelakovský and the "phyllorhize" of Chauveaud.

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REVIEW

A GERMAN TEXT-BOOK OF FOSSIL BOTANY

Handbuch der Paläobotanik, von MAX HIRMER. Band I. Thallophyta. Bryophyta. Pteridophyta. Mit Beiträgen von Dr JULIUS PIA und Dr WILHELM TROLL. Pp. 708 + xvi. Mit 817 Figuren. München u. Berlin, R. Oldenbourg, 1927. Preis geb. RM. 48.

This is an important text-book, of great interest to all students of Palaeobotany. The treatment of the different groups is full and adequate and the references well up-to-date. In the abundance and excellence of the illustrations the book compares most favourably with previous manuals of the subject.

The present volume is limited to the Cryptogams. The first section, by Dr Pia, after a sketch of the methods of preservation, deals with the Thallophyta. *Pila* and *Reinschia*, the Boghead organisms, are recognised as Algae, in spite of the grave doubts cast on their nature by some critics. The full description of the Calcareous Algae, on which the author is the highest authority, is of special interest. The great evolutionary significance of the long series of fossil Siphonaceae, revealed by Dr Pia's researches, has hardly been sufficiently recognised by botanists.

It is a pity that the author has revived the obsolete opinion that *Traquairia* was a megaspore. This genus, like *Sporocarpion*, is now admitted to belong to an independent group of organisms, probably with Rhizopod affinities.

Our knowledge of fossil fungi is still somewhat scrappy: the author rightly lays stress on the important discoveries of Devonian Fungi at Rhynie.

Dr Troll, who undertakes the Bryophyta, has a limited field. The most striking record is Mr John Walton's discovery of Carboniferous Liverworts, the first satisfactory demonstration of Palaeozoic Bryophytes.

The main part of the book, that on the Pteridophytes, is the work of Dr Max Hirmer himself. Beginning with the Devonian class of the Psilophytales, he finds their chief characteristic in the terminal sporangia, without relation to any leaves. He divides them into five families: Rhyniaceae, Horneaceae, Pseudosporochnaceae, Psilophytaceae (*Psilophyton* and *Arthrostigma*) and Asteroxylaceae. The author recognises a certain Bryophytic affinity in the first two families.

Passing on to the great class of the Lycopodiales, and beginning with the arboreal and ligulate Lepidophyta, Dr Hirmer holds that some Lepidostrobi may have been isoporous. The bare possibility may be admitted, but the evidence is inadequate. For the anatomy, *Lepidodendron vasculare* (*selaginoides*) is taken as the type, and described in detail, with fine illustrations, some of which are original. As regards *Ulodendron*, the author accepts the view that the scars mark the position of branches, adding, however, that these branches bore the cones on their ultimate branchlets. He applies a similar interpretation to *Halonia*, in which the tubercles are said in some cases to have borne the cones directly, in others on a branched shoot.

Sigillaria is treated on the traditional lines; the double foliar bundle, observed in several species, is taken as a generic character.

The interpretation of *Stigmaria* has long been a difficult problem. Dr Hirmer regards the Stigmarian branch-system at the base of the stem, as homologous with the aerial branch-system at the summit. He does not refer to the view, now commonly held, that the Stigmarian axes were of the nature of rhizophores. Among Upper Devonian Lycopods described, the most important are Archaeosigillaria, combining *Lepidodendroid* with *Sigillarian* features, and

species of *Cyclostigma*, sometimes placed in *Bothrodendron* but distinguished, among other points, by the apparent absence of a ligule.

In his general remarks on the Lycopods Dr Hirmer finds that the first evidence of their appearance is in the Middle Devonian. They manifestly attained their highest development during Palaeozoic times. *Isoetes* is regarded as the only living representative which admits of comparison with the dominant Carboniferous type. The recent *Selaginellas* are more probably derived from herbaceous Palaeozoic forms like *Selaginellites* than from the arboreal groups.

In the account of the Articulatales, the most novel feature is the discussion of the Proto-articulatae, *Hyenia* and *Calamophyton*, of Middle Devonian age, investigated by Drs Kräusel and Weyland. The dichotomous branching, a character quite foreign to the later Articulatae, is taken as the chief distinction of the early group.

In describing the well-known fructification, *Sphenophyllostachys Dawsoni*, the author speaks of a "three-armed sporangiophore." It is not clear to what he refers. No such structure has hitherto been observed in this cone.

The Calamites are included under the Equisetaceae. The account of this great class is excellent. Wherever the information is available, the stems and branches are correlated with the cones belonging to them. In his General Remarks on the whole group, Dr Hirmer discusses the vexed question of the morphology of the cone. He maintains that in most, if not all, of the bracteate fructifications, the bracts and sporangiophores are the sterile and fertile segments of the sporophyll. According to him, the scattered whorls of leaves, present among the sporangiophores in *Asterocalamites* and *Phyllothea*, have no relation to the bracts of the Calamites. He does not mention the opposite view of Lady Isabel Browne, that the bracts of Calamarian cones are a later intercalation, though this interpretation is strongly supported by the bractless fructifications of the oldest known Articulatae.

The next group, Cladoxylales, is one not usually found among the Pteridophyta. It is now more commonly included under the Cycadofilices or doubtful Pteridosperms, though the evidence for this attribution has never been very strong. Dr Hirmer puts these plants in a much humbler position, standing morphologically between the Psilophytales and Filicales, though he admits that anatomically they are remote from either. It is only quite recently, through the discoveries of Kräusel and Weyland, that we have learnt anything of the external morphology; this is now revealed in the case of the oldest known representative, the Middle Devonian *Cladoxylon scoparium* from Elberfeld. This plant had dichotomous stems, bearing small forked leaves. From anatomical evidence it appears that the long known Thuringian Cladoxylons bore large fronds. The author interprets the little leaves of the Elberfeld species as "phylloids" (in Lignier's sense) while he regards the large appendages of the later forms as true fronds derived from branch systems. In the case of *C. scoparium*, however, the "sporophylls" of Kräusel and Weyland are described as small branch systems, a view which there is little to support, they appear to be of the same nature as the vegetative phylloids.

In the great phylum of the Filicales, Arber's Primofilices are taken first, under the name Coenopteridinae. They are divided, as is now usual, into Zygopterideae, Botryopterideae and Anachoropterideae. The Zygopterids are again divided into three, the exceptional genus *Stauropteris* having a family to itself. The branching of this extremely quadriseriate frond is described as almost radial; the author suggests that there may have been no strict differentiation between leaf and stem. At present, however, the stem is unknown; Lignier doubted if there was one!

The other Zygopterids are well described on the usual lines. When the Botryopterids are dealt with, we find a statement that the stem of *Botryopteris* was freely branched. Though this may hold good for one species, *B. cylindrica*, it is evident that the author has been misled by Williamson's old description of his species, *B. ramosa*. The "branches" found by Williamson were only roots.

Kubart's discovery of the syngangic fructification of *Anachoropteris* has established the rank of this genus as the type of a family of its own. The approach to Marattiaceous organisation in this plant and in *Corynepteris* is explained as due to convergence rather than affinity.

The so-called Marattiaceae of the Carboniferous are accepted as such, without reference to Dr Kidston's suspicion that they may have been Pteridosperms and not Ferns at all: this question will probably be dealt with in the next volume. The author's point of view is indicated by his remark that the reference of disputable genera to Pteridosperms is premature, so long as no female fructification is known.

In his general remarks on the Ferns Dr Hirmer rightly points out that the leptosporangiate condition on the one hand, and the syngangic fructification on the other, may probably have been attained independently in different cycles of affinity. He holds, with Prof. Bower, that the sporangia were originally terminal, then marginal, finally becoming superficial as in most modern ferns.

The concluding observations on the comparative morphology of the Pteridophyta generally are of great interest. In discussing the nature of the leaf, the author includes the Articulatae under the microphyllous type, though their leaves are often forked. In this respect he differs from Lignier, while the general theory of that brilliant morphologist, is, as we have already seen, accepted. The thorns of *Psilophyton* and the scale-leaves of *Asteroxylon* are regarded as the precursors of the microphyllous foliage of lycopods, Psilotales and Articulatae. *Asteroxylon* to a certain extent combines both kinds of foliage, phylloids on the main stem, and incipient fronds in the form of the sporangiophores. As mentioned above, a similar interpretation is extended to the genus *Cladoxylon*.

The Psilophytales are accepted as truly primitive types, in which the higher Pteridophytic phyla have their source. This, however, must not be taken in a phylogenetic sense. Dr Hirmer expressly disclaims any rash assumption of actual evolutionary lines of descent, and confines himself to morphological comparison. All the same it is clear that he favours a monophyletic theory of the inter-relationships of the Pteridophyta.

The work of which we have given a brief sketch is, for the groups considered, the most detailed and at the same time the most attractive handbook yet published. The completion of the undertaking will be awaited with keen interest by all students of fossil plants.

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THE CLASSIFICATION OF LICHENS

By W. WATSON

PART II

THE genera which have been previously arranged into families contain the most highly developed of all the lichens in regard to thalline characters. They show various series of advances from a primitive to a complicated thalline structure. The orders Graphidales, Coniocarpales and Pyrenocarpales do not generally exhibit such diversity, and it is probably safe to assume that their consortia have been evolved, on the whole, at later periods. Before dealing with these orders further, it will be useful to repeat and place in more concise forms some of the conclusions arrived at in Part I.

(1) Every lichen species has not been formed by a separate symbiosis between a free-living fungus and alga. In a lichen formed from an original symbiosis, variation has gone on and a number of species may have been evolved. In some cases the number of derived species may have been so great, and their differentiation of sufficient magnitude, to justify the creation of genera or even families.

(2) The fungal characters are of more taxonomic importance than the algal. In sexual or asexual reproduction the cells directly concerned are of fungal origin, the function of the algal cells being nutritive.

(3) Variation and evolution are usually greater in external than in internal characters. The most constant characters are fungal; the internal characters of the apothecium, especially the spores, being those least liable to modification and selective influences by environment. There is a greater probability for a foliose thallus to be evolved from a crustaceous one than for a change of similar magnitude to occur in the spore.

(4) The presence of a simple spore in many of the fruticose and foliose lichens shows the persistence of such a character during the ages in which the thallus has been developed. The rarity of many-septate spores in the lichens, with highly-developed thalli, indicates that the origin of lichens with such spores is, generally speaking, more recent than the origin of simple-spored lichens.

(5) That mycologists do not lay great stress on the septation of the spore, for family characters, is not a strong argument against lichenologists doing so, since the fungus (in its original form) was in existence before symbiosis occurred. The formation of the thallus is a post-symbiotic development. The spore may have altered since symbiosis occurred but there is much probability that it has often remained fairly constant. The possibility of post-symbiotic change in the spore, however, shows the need of caution in excluding a particular genus from a family on account of spore-division only.

(6) Lichens with green algal cells have the greatest capacity for thalline development. This is generally better in corticolous than in saxicolous lichens, though exceptional development may occur in nitrophilous habitats. The thallus, though often more immersed in calcareous rock, is usually better developed than that of a lichen on siliceous rock, especially when the latter is hard. Terricolous lichens, owing to their insecurity of tenure, are often little developed.

(7) Paraphyses are fairly constant and must be considered in lichen taxonomy. Their disappearance, coherence, colour and degree of swelling at the apices, and intrusion of algae among them, do not seem to be of great significance for family characters.

(8) The symbiotic relationship, and also to some extent the habitat, affects the algal cells. The appearance presented by the algal cells in the thallus is often very different from that presented by the free-living alga. In consequence there is some confusion and conflict of opinion as to the name given to the algal symbiont. This difficulty is often avoided in the text by simply giving the colour of the algal cells. There is much uncertainty as to the exact relationship of *Trentepohlia* with the allied algae (*Phyllactidium*, etc.) found in epiphyllous lichens.

(9) So much uncertainty exists as to the exact relationship of the fungal symbiont that definite naming has been avoided.

(10) Substitution of one alga for another has probably occurred in some cases.

(11) Spermatogonia and spermatia are too uncertain, both in nature and occurrence, to be held of great taxonomic importance.

Order GRAPHIDALES

As Lorrain Smith (1926) p. 278) writes: "there is ample evidence of polyphyletic descent in the series." A natural classification should attempt to arrange those having a common origin in the same group, and the arrangement into families, as given by Zahlbruckner, requires some alteration. Due importance is usually attached to the apothecium and the spore except in the family Graphidaceae. In Roccellaceae and Dirinaceae the ascus contains 8 septate spores and the thallus has *Trentepohlia* algae. Chiodectonaceae is similar in these respects except that the ascus may contain fewer spores and that *Heterothallus* or *Phyllactidium* is occasionally present in the thallus instead of *Trentepohlia*. In Arthoniaceae the spores are also 8 in the ascus and septate but the thallus in two genera (*Allarthonia* and *Allarthonothelium*) contains green algal cells. In Lecanactaceae the spores are septate¹, 8 or less in the ascus, and the algal cells are *Trentepohlia*. This family is not included in this group by Zahlbruckner, though he acknowledges its relationship as follows: "die verwandtschaftlichen Beziehungen der *Lecanactidaceae* zu den *Graphaceae* sind sehr nahe; die Selbständigkeit der Familie innerhalb der *Cyclocarpineae* würde von Wainio begründet" (1908 p. 131). Lorrain Smith includes it with the group and, if the family is retained, is certainly justified in doing so when *Platygrapha* (*Schismatomma*) is considered as one of its genera, as is done by both Zahlbruckner and herself. When the lirelloid members of *Schismatomma* are distributed in their respective positions amongst the Graphidales there seems little reason for retaining the family. *Pseudolecanactis* and *Catinaria* may be considered as derived from *Lecidea* and *Catillaria* respectively, by the substitution of *Trentepohlia* for a protococcoid alga in the thallus, whilst *Lecanactis* and some *Schismatommae* may be substitution products of *Bilimbia* and *Bacidia*. *Melampyrium* is not described in the same way by different lichenologists but seems near to *Rhizocarpon* with *Trentepohlia* for its algal symbiont.

The five families given by Zahlbruckner are primarily dependent on apothecial characters, except in Roccellaceae, which is distinguished from the others owing to the fruticose habit of its members. Arthoniaceae is distinguished from the other families with a crustaceous thallus by the immarginate apothecia; Dirinaceae and Graphidaceae have marginate apothecia, the thallus being corticate

¹ *Pseudolecanactis*, with simple spores, is included in the family by Zahlbruckner.

or non-corticate respectively; whilst Chiodectonaceae has the apothecia aggregated in special portions (stromata or pseudostromata) of the thallus. However convenient this method of classification may be from a collector's point of view (since it provides him with an easy way of arranging his plants so as to determine them readily), it is too artificial unless other characters are in conformity with it. There is no more reason for placing all the fruticose plants, simply because they are fruticose, in one family than there is for putting in a special family all the woody plants belonging to the Rosaceae or Papilionaceae, or for creating a special family for *Cytisus*, *Robinia* and other arborescent Papilionaceae. Again, the formation of the family Chiodectonaceae, on account of the aggregation of the apothecia, is no more justifiable than the creation of a special family to receive *Trifolium* from Papilionaceae and *Poterium* from Rosaceae. It is a method which is not adopted in other orders of lichens except in the Pyrenocarpaceae. Even in the Graphidales it is not adopted in regard to *Synarthonia*, a stromatoid *Arthonia*, which is allowed to remain in the Arthoniaceae. The aggregation of the apothecia has occurred independently in various genera, and the various members of Chiodectonaceae should be distributed amongst the other families, in accordance with their probable derivation. During the aggregation the apothecial margin is likely to become less prominent, or entirely disappear, but in some cases (e.g. *Glyphis*, *Sarcographa*, *Sarcographina*) it remains permanent. In the following table the presence or absence of a margin is neglected and the arrangement shows that Chiodectonaceae is a variable group in regard to septation, colour and cell-shape of spores and paraphyses, also that its various genera agree, in these respects, with genera belonging to other families of Graphidaceae. This agreement generally extends to other characters as well. Three members are without definite parallel representatives. *Rotularia* may be a derivative of *Mazosia*, *Enterostigma* of *Sclerographis* with longitudinal septa added in the spore, and the only genus without a known representative \pm parallel to it is *Enterodictyon*. The spore-cells of this approach those of the *Opegrapha* group, but the simple and free paraphyses indicate a relationship to *Graphis*.

In the Graphidaceae of Zahlbruckner there are three groups: (1) the main group in which the spores are septate, up to 8 in the ascus and the thallus contains trentepohlioid algae; (2) a small group in which the spores are simple, or almost so, and many in the ascus; (3) a group in which the ascus contains 8 (or less) simple (occasionally

Genera of Chiodectonaceae compared with parallel genera of Graphidales with single apothecia.

Apothecia grouped (Chiodectonaceae)	Apothecium single	Spores					Algal symbiont
		Septation	Colour	Shape of cells	No. in ascus	Paraphyses	
Glyphis	Graphis	3-11 transverse	o	± lens-shaped	4-8	Simple and free	Trentepohlia
Pycnographa	Micrographa	1 transverse	o to brownish	Unequal	8	Simple and free	Phyllactidium
Chiodecton	Opographa	Many transverse	o	± cylindrical	8	Branched and anastomosing	Trentepohlia
Rotularia	—	Many transverse	o	"	8	Branched and anastomosing	Heterothallus
Mazosia	Fouragea	Many transverse	o	"	4-8	Branched and anastomosing	Phyllactidium
Sarcographa	Phaeographis	Many transverse	Dark	± lens-shaped	—8	Simple and free	Trentepohlia
Sclerophyton	Sclerographis	Many transverse	Dark	± cylindrical	—8	Branched and anastomosing	"
Medusulina	Graphina	Muriform	o	± rounded	1-8	Simple and free	"
Enterodictyon	—	"	o	± cubic	—8	"	"
Sarcographina	Phaeographina	"	Dark	± rounded	1-8	"	"
Minksia	Dictyographa	"	o	± cylindrical	1-8	Branched and anastomosing	"
Enterostigma	—	"	Dark	"	—8	Branched and anastomosing	"

r-septate) spores, and the thallus usually possesses green algal cells. The last group corresponds more-or-less to Xylographidaceae in Mudd's *Manual* and to Reinke's Xylographacei, though in these groups the sporal characters were not considered as diagnostic, so that the name of Xylographaceae may be used for it. The second group corresponds to the family Acarosporaceae of the Parmeliales and may be called, after one of its members, Graphinellaceae. The main group cannot be considered as a simple group as two kinds of spore-cells are shown and the paraphyses are different. In one of these smaller groups the cells of the spore are cylindrical or cubic and the asci are accompanied by branched and anastomosing paraphyses; in the other the spore-cells are lens-shaped or rounded with thick walls and the paraphyses are simple and free. The following table shows the main characters of the members of Zahlbruckner's Graphidaceae inclusive of the lirelliform members of Roccellaceae. The algal cells are *Trentepohlia* unless otherwise stated¹.

The group to which *Graphis* belongs has spores with lens-shaped or rounded cells, usually three or more in number, and having thick walls²; the paraphyses are simple and free and the hypothecium is colourless or almost so. The name of Graphidaceae is restricted to this group. The *Opegrapha* group has spores with more-or-less cylindrical cells, usually three or more in number, and having thin walls; the paraphyses are branched and anastomosing, the hypothecium is often dark and the apothecia are usually more superficially situated. This group constitutes the emended family Opegraphaceae. *Melaspilea*, *Micrographa* and the stromoid *Pycnographa* form a group, which may be an offshoot from the line which gave rise to the Graphidaceae, or may have an independent origin. The group is however sufficiently distinct to form the separate family of Melaspileaceae.

Family ROCELLACEAE

The relations of this family, as given by Zahlbruckner, are shown in the table on page 92.

There are three members with lirelliform apothecia in which a thalline margin is absent. *Ingaderia* and *Reinkella* agree almost exactly with *Opegrapha* except for their fruticose habit. *Roccellographa*, even though it has an immersed apothecium, also seems to

¹ The usual interpretation of the green algal cells as *Palmella* has been followed.

² These characters are also considered to be of taxonomic importance in the Pyrenocarpaceae. See p. 99.

Comparative table of Graphidaceae (sec. Zahlbruckner and inclusive of lirelliform Roccellaceae).
Thallus crustaceous and algal cells Trentepohlia unless otherwise stated.

Spore			Hypothecium	Paraphyses and any other special characters	
Septation	Colour	Cell-shape			
Parallel	o	± lens-shaped	Pale	Simple and free	Graphis
"	o	"	"	Simple and free with clavate and verrucose apices	Acanthographis
"	Dark	"	"	Simple	Phaeographis
"	o	"	"	"	Graphina
"	Dark	"	"	Simple with clavate and verrucose apices	Acanthographina
"	"	"	"	Loose and relatively thick. Algal cells Palmella?	Phaeographina
"	"	"	Brownish	Branched and anastomosing	Xyloshistes
Parallel	o	± lens-shaped to cubic	Pale	"	Helminthocarpon
"	o	± cylindrical	Dark or pale	Branched and anastomosing. Alga Phylactidium	Opegrapha
"	3-9	"	Pale	Branched and anastomosing. Algal cells Palmella	Fouragea
"	o	"	"	Branched. Thallus fruticose	Aulaxina
"	o	"	Dark	Branched and coherent. Thallus fruticose	Ingaderia
"	o	"	"	"	Reinkella
"	Dark	"	Pale	—	Gymnographis
"	"	"	Dark or pale	Branched and anastomosing. Apothecia immersed	Sclerographis
"	"	"	Pale	Branched and anastomosing. Pal-mella. Compound hymenium	Roccellographa
"	"	"	"	Simple and free. Alga Phylactidium	Dictyographa
"	"	"	Dark	Branched and coherent. Algal cells Palmella	Diplogramma
"	o	"	Dark	Branched and coherent. Algal cells Palmella	Micrographa
"	o	"	Pale	Simple and free. Algal cells green	Nelaspila
"	o	"	Dark	Compound hymenium. Algal cells Palmella	Encephalographa
"	o	"	"	"	Lithographa
"	o	"	"	"	Xylographa
"	o	"	"	"	Psychographa
Simple	o	Elongate	Dark	Branched and anastomosing	Spirographa
"	o	"	Pale	Simple and free	Graphinella

Spores many in ascus.

Comparative table of Roccellaceae (sec. Zahlbruckner).
 Thallus fruticose (except in Roccellina where it is crustaceous) with Trentepohlia.
 Ascus with 8 parallel- and transversely-septate spores. Paraphyses branched.

Septa	Spore		Apothecium				Hypothecium	
	Colour	Shape	Position	Shape	Margin			
7-8	o	Fusiform	Sessile	Lirelliform	Proper	Dark	Ingaderia	
3	o	"	±elevated	Rounded	Thalline	"	Dendrographa	
3	o	"	Sessile	"	Proper	"	Roccellaria	
2, median cell small	Brownish	Oval	Adnate	"	Thalline	Pale	Darbshirella	
5-7	"	—	Immersed	Lirelliform	Proper	Colourless	Roccollographa	
-7	o	Cylindrical	Sessile	"	Thalline	Dark	Reinkella	
3	o	Fusiform	"	Rounded	Proper or thalline	"	Roccellina	
3-	o	—	"	"	±thalline	"	Roccella	
3	o	Fusiform	±sessile	"	Thalline	Pale	Combea	
3	o	"	±elevated	"	±thalline	"	Pentagenella	
3	Brownish	"	±sessile	Rounded and ±lobed	"	Dark	Schizopelte	
3	"	Cylindrical	Shortly stalked	Rounded and ±lobed	"	"	Simonyella	

In Ingaderia, Dendrographa, Roccellaria and Darbshirella the hyphae are parallel to the surface of the thallus, whilst in the others they are transverse.

have been derived from *Opegrapha* through *Sclerographis*. These three genera may then be considered as fruticose members of Opegraphaceae. After these have been removed from Roccellaceae the remaining genera have rounded apothecia and there seems little reason for retaining the emended family amongst the Graphidales.

Family DIRINACEAE

Characters and genera as given by Zahlbruckner. *Cyclographa* differs from *Dirina* and *Dirinastrum*, the other two genera belonging to this family, in having the paraphyses branched and coherent instead of simple and free. The roundish apothecia, which are usually provided with a thalline margin, and the corticate nature of the thallus, indicate the near relationship of this family to the Parmeliales.

Family ARTHONIACEAE

In this family the immarginate apothecium is taken as a critical character. However useful it may be for determinative purposes, it cannot, by itself, be regarded as a critical taxonomic character. In many genera (e.g. *Lecidea*) belonging to the Parmeliales, the presence or absence of a proper margin to the apothecium is disregarded. Even in the Graphidales some species belonging to other families have forms in which the proper margin is absent, e.g. *Opegrapha herpetica* form *arthonoidea*, *O. atra* var. *arthonoidea*, forms of *O. varia* and *O. siderella*. The immarginate apothecium is, however, found in conjunction with other characters which are rare or absent in other families, so that Arthoniaceae may be considered as a fairly natural group. The asci are usually short and pyriform, the paraphyses are usually branched and closely coherent and the spore is often unequally celled. The latter character is not shown in all the species and is occasionally found in members of other families, e.g. *Encephalographa*, *Micrographa*, *Darbishirella*, *Pycnographa* and some species of *Opegrapha*.

The characters and genera are as given by Zahlbruckner.

Comparative table of Arthoniaceae

		Algal cells present in thallus		
		Palmella	Trentepohlia	Phyllactidium
Spore with parallel septa	Apothecium single	Allarthonia	Arthonia	Arthoniopsis
	Apothecia grouped	—	Synarthonia	—
Spore muriform	Apothecium single	Allarthothelium	Arthothelium	Trichophyma

Family XYLOGRAPHACEAE

Thallus crustaceous, non-corticate, fastened to the substratum by hyphae, epi- or hypo-phloeodal, with green algal cells ("*Palmella*"). Apothecia oblong or ovoid or more-or-less angular, simple or branched, sessile or erumpent, marginate; ascus more-or-less clavate, with 4-8 simple (or 1-septate), colourless (or dark) spores.

Encephalographa is peculiar in some respects, such as the 1-septate brown spores with unequal cells. It is almost distinct enough to form a separate family, though it is possibly derived from *Lithographa*.

Spores simple, colourless and ellipsoidal.

Apothecium with simple hymenium.

Hypothecium dark *Lithographa*

Hypothecium pale *Xylographa*

Apothecium with 2-4 parallel hymenia ... *Ptychographa*

Spores 1-septate and brown. Apothecia usually

grouped *Encephalographa*

Family MELASPILEACEAE

Thallus crustaceous, non-corticate, fastened to the substratum by hyphae, epi- or hypo-phloeodal, with trentepohlioid algal cells. Apothecia lirelliform or roundish, single or congregate, marginate; paraphyses simple and free; ascus more-or-less clavate with 8 (or fewer) 1-septate (occasionally 3-septate), colourless or dark spores.

There is a possibility that this group has been derived from *Xylographa* and that from the same evolutionary line the family Graphidaceae (in its restricted sense) has been derived.

Algal cells *Trentepohlia*. Spores becoming darker ... *Melaspilea*

Algal cells *Phyllactidium*.

Apothecia in stromata *Pycnographa*

Apothecia not in stromata *Micrographa*

Family OPEGRAPHACEAE

Thallus crustaceous, rarely fruticose, usually with trentepohlioid algal cells. Apothecia usually lirelliform, simple or branched, sessile and often more-or-less superficial (exceptionally immersed), single or congregate or in stromata. Ascus more-or-less clavate, 8- or fewer-spored. Paraphyses branched and anastomosing. Spores usually colourless, with 3 or more parallel transverse septa, or muriform; cells more-or-less cylindrical with thin walls.

Spores transversely (3-9)-septate.

Algal cells *Trentepohlia*.

Thallus fruticose.

Hyphae parallel to surface.

Hypothecium dark. Spores colourless.

Soredia absent *Ingaderia*

Hyphae perpendicular to surface.

Apothecia sessile. Hypothecium dark.

Spores colourless. Soredia present ... *Reinkella*

Apothecia immersed. Hypothecium pale.

Spores dark. Soredia absent *Roccellographa*

Thallus crustaceous.

Spores 3-septate brown. Hypothecium

pale. Apothecia immersed *Gymnographa*

Spores 3- or more-septate. Apothecia usually superficial.

Spores colourless.

Apothecia not in stromata *Opegrapha*

Apothecia in stromata.

Hypothecium pale *Enterographa*

Hypothecium dark *Chiodecton*

Spores dark.

Apothecia not in stromata *Sclerographis*

Apothecia in stromata *Sclerophyton*

Algal cells *Phyllactidium* (or *Heterothallus*). Spores colourless.

Apothecia not in stromata *Fouragea*

Apothecia in stromata.

Algal cells *Phyllactidium* *Mazosia*

Algal cells *Heterothallus* *Rotularia*

Algal cells "*Palmella*." Spores colourless.

Spores 3-8-septate. Hypothecium pale. Hy-

menium simple *Aulaxina*

Spores 3-septate. Hypothecium dark. Hy-

menium compound *Diplogramma*

Spores muriform. Algal cells *Trentepohlia*.

Apothecia not in stromata *Dictyographa*

Apothecia in stromata.

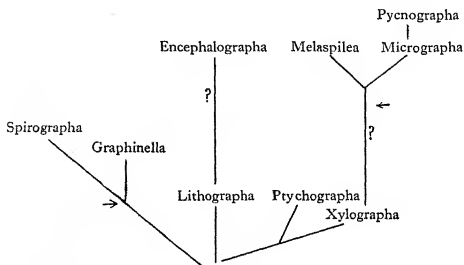
Spores colourless *Minksia*

Spores dark *Enterostigma*

Family GRAPHIDACEAE

Thallus crustaceous, cortex absent or amorphous, epi- or hypophloeodal, fastened to the substratum by hyphae, with *Trentepohlia*

or rarely *Palmella*. Apothecia usually more or less immersed, lirelliform or roundish, simple or branched, single or aggregate, usually marginate. Ascus \pm clavate, with 8 or fewer spores. Paraphyses simple and free. Hypothecium usually colourless or pale. Spores colourless or brown, with 3 or more parallel transverse septa or muriform; cells short, lens-shaped or rounded with thick walls.



Helminthocarpon is a rather peculiar genus. The branched and coherent paraphyses link it to Opegraphaceae, but the shape of the spore-cell is a more important taxonomic character, and this is nearer to that of Graphidaceae. The volva-like thalline margin is also peculiar. The stromoid *Enterodictyon* has the spore-cells more cubic and with thinner walls, but its paraphyses are simple and free. There is no known member of Opegraphaceae parallel to it and, on the whole, it agrees better with Graphidaceae, though its derivation is doubtful.

Spores transversely (3-19)-septate.

Spores colourless.

Paraphyses clavate and warted at apices *Acanthographis*

Paraphyses little clavate and smooth at apices.

Apothecia not in stromata *Graphis*

Apothecia in stromata *Glyphis*

Spores brown or dark.

Apothecia not in stromata *Phaeographis*

Apothecia in stromata *Sarcographa*

Spores muriform.

Spores colourless. Paraphyses simple and free.

Paraphyses clavate and warted at apices *Acanthographina*

Paraphyses little clavate and smooth at apices.

Apothecia not in stromata *Graphina*

Apothecia in stromata.

Spore-cells lens-shaped with thick walls *Medusulina*

Spore-cells \pm cubic with thinner walls *Enterodictyon*

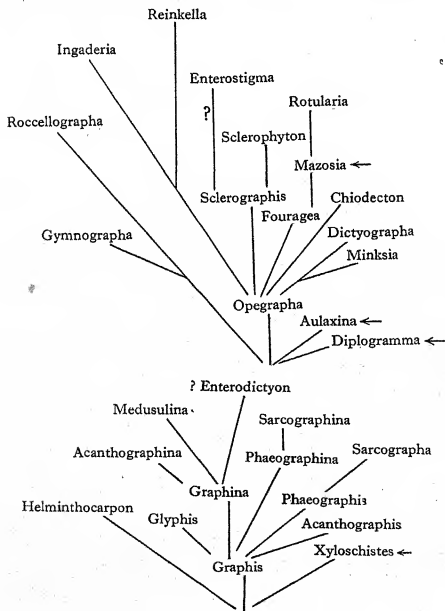
Spores colourless. Paraphyses branched and coherent *Helminthocarpon*

Spores dark. Hypothecium brownish. Algal cells green *Xyloschistes*

Spores brown. Algal cells *Trentepohlia*.

Apothecia not in stromata *Phaeographina*

Apothecia in stromata *Sarcographina*



Family GRAPHINELLACEAE

Thallus crustaceous, non-corticate, fastened to the substratum by hyphae, with *Trentepohlia* algal cells. Apothecia lirelliform, single, superficial or immersed. Ascus many-spored. Spores colourless, simple or indistinctly 1-septate and usually elongate.

Paraphyses branched and anastomosing	<i>Spirographa</i>
Paraphyses simple and free	<i>Graphinella</i>

Order CONIOCARPALES

The families and genera given by Zahlbruckner are provisionally accepted, though there are some indications that a revision of the order, in which greater importance is given to sporal characters, is desirable. *Tylophorella*, with its many-spored ascus, seems comparable to *Acarospora* of the Parmeliales and to *Graphinella* in the Graphidales. The family Cypheliaceae, which differs chiefly from the family Calicaceae in the absence of an apothecial stalk is not used by Lorrain Smith (32). In some of the genera placed in Calicaceae the apothecia are sessile or almost so (*Sphinctrina*, *Pyrgidium*), whilst in some (*Pyrgillus*, *Tylophoron*) placed in Cypheliaceae the apothecia are more-or-less elevated. The length of the apothecial stalk is a variable quantity in the species in which it is usually present, and scarcely warrants having such taxonomic importance attached to it.

Order PYRENOCARPALES

The classification into families is again largely influenced by its convenience to the collector and determinator. From this point of view it is a successful one, as it renders a pyrenocarpous lichen easy to place in its particular group. This influence has, however, been somewhat detrimental to a natural classification and has tended to exaggerate the importance of thalline and other characters easily seen by the naked eye, or with the aid of a lens.

The development of a foliose thallus is chiefly shown in those Pyrenocarpaceae with green algal cells. It is considered as the critical character on which the family Dermatocarpaceae is founded, whilst the fruticose habit distinguishes the family Pyrenothamniaceae. These two "families" are varied in regard to spores and paraphyses; they do not form natural groups, and it seems probable that the

genera composing them have originated independently from genera with crustaceous thallus and corresponding thecial characters. In some cases the genera are of comparatively recent creation: thus *Agonimia* was founded in 1909 to receive the more-or-less foliose species of *Polyblastia*, whilst *Nylanderella* was created as late as 1914. The only British representative of *Agonimia* is still given as *Polyblastia tristicula* in the standard Monograph of British Lichens. It was found by Admiral Jones in 1864 at Aviemore and that was the only British record till it was discovered in Somerset in 1917. All the characters agree with *Polyblastia* and its foliose nature is often little more pronounced than in some specimens of *Bilimbia sabuletorum*, so that even its generic segregation is doubtful, whilst its relegation to another family is unwarranted. The following table shows the relationships of the genera placed in Dermatocarpaceae and Pyrenothamniaceae, with those placed in Verrucariaceae. It indicates that the foliose and fruticose genera should be regarded as derived from various sources and that the retention of the families Dermatocarpaceae and Pyrenothamniaceae is phylogenetically unsound.

The spores in species of *Verrucaria* are remarkably constant in regard to their simple character, and the same statement holds good for other genera with simple spores. The unicellular character of the spore can therefore be taken as of considerable systematic value. There is much less constancy in the septate spores. In the genus *Thelidium* one and the same species may show what appear to be mature spores with different septation, some 1-septate, others 3-septate, whilst occasionally a longitudinal septum connects two of the transverse septa. The continued division, so that the spore becomes muriform, is shown in the subdivision *Thelidioides* (37) of *Polyblastia*. Some spores in the ascus of one and the same perithecium are 1-septate, some 3-septate, in others further transverse septa occur, whilst, finally, oblique or longitudinal septa appear and the spore becomes muriform. These changes may, of course, be recapitulations of the fungal ancestor antecedent to its symbiosis with the algae. Whether antecedent or not, the gradual transition from one to further septa in different Pyrenocarpales implies that such septation must be used cautiously, even for generic distinction.

The shape of the sporal chambers seems to be of greater value than their number. Most Pyrenocarpales have cylindrical or cubic cells with thin walls, but some genera possess lens-shaped or \pm rounded cells with thick walls. This difference is used for generic distinction

Comparative table of Pyrenocarpaceles with green algae and simple apothecia with apical ostioles.

Septa	Spore			No. in ascus	Paraphyses	Hymenial algae	Crustaceous	Foliose	Fruticose
	Colour	Shape							
None	o	± ellipsoid		8 (-16)	Mucilaginous and disappearing	o	Verrucaria	Dermatocarpon	—
"	o	Vermiform		8	Mucilaginous and disappearing	o	Sarcopyrenia	—	—
"	o	± ellipsoid		Many	Mucilaginous and disappearing	o	Trimmatothele	—	—
"	o or brownish	"		1	Branched	Present	Thelidopsis	Anapyrenium	—
"	o or brownish	"		4-8	"	o	Thrombium	—	—
1-3	o	"		8	Mucilaginous and disappearing	o	Thelidium	Placidopsis	Nylanderella
1-3	Brown	"		8	Mucilaginous and disappearing	o	—	Heterocarpon	—
1	"	"		8	Simple with mucilaginous walls	o	Thelidopsis	—	—
5-7	o or brownish	Elongate		6-8	Mucilaginous and disappearing	o	—	Normandina	—
3	o	Fusiform		8	Branched	o	Geisleria	—	—
Many	o	Acicular		4-8	Unbranched	o	Gongylia	—	—
Many	o or dark	± ellipsoid		1-8	Mucilaginous and disappearing	o	Polyblastia	Agonimia	Pyrenothamnion
"	"	"		1-8	Mucilaginous and disappearing	Present	Staurothete	Endocarpon	—
"	o	"		2-8	disappearing	o	Microglana	Psoroglaena	—
"	o or pale	± fusiform		4-6	Simple and ± free	o	Aspidothelium *	—	—
Many	o	Fusiform		—	Branched and coherent	o	Aspidopyrenium *	—	—

* Perithecium widening to form a shield round the ostiole.

and, in conjunction with other characters, must be considered for the grouping into families. The paraphysal characters are also fairly constant in certain groups and due consideration must also be given to them. The paraphyses sometimes become mucilaginous and disappear, but this character appears to have been evolved independently in several families and even in different genera. The darkening of the spore and the expansion of the apothecial apex to form a shield have also occurred independently in different consortia. The formation of stiff hairs on the perithecium of *Stereochlamys* and *Trichothelium* is probably a case of convergence, though there is a slight possibility that both genera were derived from the same ancestral form possessing a bristly perithecium. In both the ascus has 8 colourless spores, hymenial algae are absent and the paraphyses are simple. *Trichothelium* occurs on leaves on living trees, the alga is *Phyllactidium*, the spores are many-septate and it is probably evolved from *Phylloporina*. *Stereochlamys* occurs on bark, the alga is *Trentepohlia*, the spores are muriform and it seems allied to *Pyrenula*.

Algal cells are present in the hymenium of *Staurothele*, *Endocarpon* and *Thelenidia*, but their occurrence is of little phylogenetic significance as their intrusion seems to have been quite independent in the first and last of these genera.

The following tables show the characters of the genera placed in the Pyrenocarpaceae. Some genera, as *Coriscium* and *Cocciscia*, are omitted because of the unknown, or doubtful, nature of some of the characters.

COMPARATIVE TABLES OF PYRENOCARPALES

Table I. Spores simple. No stromoid representatives.

Spore			Thallus			
Colour	Shape	No. in ascus	Paraphyses	Alga	Crustaceous	± foliose
o	± ellipsoid	8	Disappearing	Green	Verrucaria	Dermatocar
o	Vermiform	8	"	"	Sarcopyrenia	—
o	Elongate	8	± disappearing	Prasiola	Mastodia	—
o	± ellipsoid	Many	Disappearing	Green	Trimmatothele	—
o	"	"	Simple and free	Dactylococcus	—	Placothelium
o	"	6-8	Almost simple	Trentepohlia	Coccotrema	—
o	Elongate	6-8	Delicate and short	Nostoc	Hassea	—
o	± ellipsoid	8	Branched	"	Rhabdospora	—
o	"	-8	± branched	Scytonema	Rhodothrix	—
o	"	1	Branched (hym. algae)	Green	Thelenidia	—
o	"	4-8	± branched	"	Thrombium	—
Brown	"	4-8	"	"	"	Anapyrenium
o	"	8	± branched and anastomosing	Trentepohlia	—	Lepolichen
o	"	2-4	Branched and anastomosing	"	Monoblastia	—
Dark	"	8	Branched and anastomosing	Phyllactidium	Haplopyrenula	—

Table II.
Spores transversely septate. No stromoid representatives.

Septa	Colour	Spore		Cell-form	Paraphyses	Algal cells	Thallus	
		No. in ascus	Shape				Crustaceous	± foliose or fruticose
1	o	8	Fusiform	± cylindrical	Disappearing	Scytonema or Sirosporon	Eolichen	—
3	Brown	4	± ellipsoid	"	"	Nostoc	—	Pyrenidium (fr.)
1-3	o	8	"	"	± disappearing	Green	Thelidium	Placidopsis
1-3	Brown	8	"	"	Disappearing	"	—	Nylanderella (fr.)
1	Dark	8	"	"	Simple with slimy walls	"	Thelidiopsis	Heterocarpon
5-7	o (brown)	6-8	Elongate	"	Disappearing	"	—	Normandina
1(-5)	Brown	4-8	± ellipsoid	"	Branched and anastomosing	Trentepohlia	Microthelia	—
1-3	"	8	"	"	Branched and anastomosing	Phyllactidium	Microtheliopsis	—
1	o	8	"	"	Branched and entangled	Trentepohlia	Acrocordia	—
3	o	8	"	"	Branched	Green	Geisleria	—
1	o	8	"	"	± branched	Xanthocarpa	Xanthopyrenia	—
1	o	Many	Cylindrical	"	"	Green	Epigloea	—
(0)-3	o	"	± ellipsoid	"	Simple	Trentepohlia	Thelopsis	—
(0)-	o or brown	4-	"	"	—	Green and colonial	Moriola and Speiconisca	—
more	many	many	Acicular	"	Simple	Green	Gongylia	—
Many	o	4-8	Fusiform	± lens-shaped	Branched and coherent	"	Aspidopyrenium (perithecium shield-forming)	—
"	o	8	"	"	"	"	"	—

Table III.

Spores transversely septate. Stromoid representatives sometimes present. Thallus crustaceous except in Strigula.

Sta	Spore			Paraphyses	Alga	Apothecium single	Apothecia stromoid or compound	Apothecia oblique or with oblique mouth	Apothecia radiate and ±stromoid
	Colour	No. in ascus	Shape						
5	0	-8	±ellipsoid	Branched and anastomosing or 0	Trentepohlia	Arthopyrenia	Tomasella	Pleurotrema	—
5	0	8	"	Branched and anastomosing or 0	Phyllactidium or Heterothallus	Raciborskiella	—	—	—
any	0	-8	Elongate	Branched and anastomosing or 0	Trentepohlia	Pseudosagedia	Athrismidium	Pleurotrema (p.p.)	—
any	0	4-8	Acicular	Branched and anastomosing or 0	"	Leptorhaphis	Celothelium	Pleurotrema (p.p.)	—
ly	0	4-8	"	Simple and free	"	Belonia	—	—	—
	0	8	"	Disappearing	Phycopeltis	Phylloblastia	—	—	—
5-	Brown	6-8	Elongate	Simple and free	Trentepohlia	Blastodesmia	—	—	—
	0	8	"	"	"	Porina	—	—	—
	0	8	"	"	Phyllactidium or Heterothallus	Phylloporina	—	—	—
y	0	8	"	"	Phyllactidium	Trichothelium	—	—	—
	o-brown	8	Unequal	Simple or branched	Trentepohlia	—	Asteroporum	—	—
	"	8	Elongate	±branched and coherent or 0	"	—	Mycoporellum	—	—
-	Brown	8	Fusiform	Simple and free	"	Pyrenula	Melanotheca	—	—
-	"	-8	±fusiform	Branched and anastomosing	"	—	"	Parathelium	Pyrenastrum
	o-brown	8	Fusiform	Absent	Phycopeltis	Micropyrenula	—	—	—
-	0	-8	±fusiform	Branched and anastomosing	Trentepohlia	Pseudopyrenula	Trypethelium	Plagiotrema	Astrothelium
	0	8	Elongate	Simple and free	Phyllactidium or Heterothallus	Strigula*	—	—	—

* In Strigula the thallus is radiately-lobed at the margin.

Table IV.
Spores muriform. Stromoid representatives sometimes present.

	Spore		Paraphyses	Algal cells	Apothecium single. Thallus crustaceous	Apothecium single. Thallus foliose or fruticose	Apothecia stromoid or compound	Apothecia oblique or with oblique mouth	Apothecia radiate and stromoid
	No. in ascus	Cell-form							
colour									
r dark	1-8	± cylindrical	Disappearing	Green	Polyblastia	Agoutimia	—	—	—
r dark	1-8	"	Disappearing	"	Staurothela	Endocarpon	—	—	—
rown	1-8	"	Disappearing	"	—	Pyrenothamnium (fruticose)	—	—	—
o	2-8	"	Branched	"	Microglauca (p.p.)	—	—	—	—
rown	2-8	"	"	"	Microglauca (p.p.)	Psoroglaena	—	—	—
dark	8	"	"	Scytonema	Pyrenothrix (p.p.)	—	—	—	—
o	1-8	± cylindrical or cubic	Branched and anastomosing	Trentepohlia	Polyblastopsis (p.p.)	—	Laurera	Campylothelium	—
rown	1-8	± cylindrical	Branched and anastomosing	"	—	—	Bottaria (p.p.)	Pleurothelopsis	Parmentaria
brown	6-8	± cubic	Branched and coherent or o	"	—	—	Mycoporium	—	—
o	—8	± cylindrical or cubic	Simple and free	"	Clathroporina	—	—	—	Cryptothelium (p.p.)
rown	1-8	± cylindrical	"	"	—	—	—	—	Cryptothelium (p.p.)
o	4-8	Cubic	"	Phyllactidium	Phyllobathelium	—	Bottaria (p.p.)	—	—
rown	1-8	Lensiform	"	Trentepohlia	Anthracotheleum	—	—	—	—
o	8	± cylindrical	"	"	Stereoclamys*	—	—	—	—
r pale	4-6	± cubic	"	Green	Aspidothelium†	—	—	—	—

* Stiff hairs on upper part of perithecium.
† Perithecium expanding to form a shield around the ostiole.

The stromoid pyrenocarpous lichens are often grouped together under a distinct family, though Wainio distributed them as sub-genera under the corresponding genera of Pyrenulaceae. Zahlbruckner rejects the method of Wainio, but gives insufficient reasons for grouping them under the special family Trypetheliaceae. As with Chiodectionaceae amongst the Graphidales, they are here recognised as constituting different genera, and placed in their appropriate positions in other families. It may also be noted that stromata or pseudostromata are met with in some of the species placed by Zahlbruckner in Astrotheliaceae, whilst the perithecia are compound in Mycoporaceae and often congregate in plants belonging to genera placed in Pyrenulaceae, e.g. species of *Arthopyrenia*, *Leptorhaphis*, *Pseudopyrenula*, *Anthracotheceum*. In those included in the special family Trypetheliaceae, *Tomasiella* is a stromoid genus corresponding to *Arthopyrenia*, *Athrismidium* (used as a sub-genus) to *Pseudosagedia* (sub-genus of *Arthopyrenia*), *Celothelium* to *Leptorhaphis*, *Melanotheca* to *Pyrenula*, *Trypethelium* to *Pseudopyrenula* and *Laurera* to *Polyblastiopsis*. *Bottaria* seems to correspond to a compound *Pleurotheliopsis* with a vertical mouth. The corticate nature of the upper part of the thallus suggests that it may have developed from a lichen with green algae, but the only one it could directly develop from is *Microglæna*, and in this the cortex is doubtful. Another possibility is that it comes from *Laurera*, but the varying character of the paraphyses indicates that it is a mixed genus.

The family Strigulaceae is founded largely on habitat. Most of the genera are epiphyllous and contain a trentepohlioid alga other than *Trentepohlia*. The apothecia are always single and vertical with straight ostiole, but the sporal and paraphysial characters are too varied for the genera to be considered as a phylogenetic group. The family is a useful one for determinative purposes but, on phylogenetic grounds, the genera appear to be better distributed as derivatives of genera belonging to other families. Except for the alga and habitat, *Microtheliopsis* corresponds to *Microthelia*, *Phylloporina* to *Porina*, *Phyllobathelium* to *Clathroporina*, *Raciborskiella* to *Pseudosagedia*, and *Haplopyrenula* to *Monoblastia*. *Micropyrenula* is similar to *Pyrenula* and *Phylloblastia* to *Belonia*, though paraphyses are absent or disappear in the epiphyllous plants. *Trichothelium* is like *Phylloporina* but possesses hairs on the perithecium, whilst *Strigula* itself is also like a *Phylloporina*, which forms definite circular patches with a radiate margin.

Family MORIOLACEAE

Characters and genera as given by Zahlbruckner (³⁶) p. 64). *Bacotillhis*, given as a section or sub-genus of *Spheconisca*, may be given generic rank because of its many-spored ascus and its simple or indistinctly septate spores.

Family EPIGLOEACEAE

Characters and genus as in Zahlbruckner (³⁶) p. 65).

Family VERRUCARIACEAE

Thallus crustaceous or foliose, non-corticate when crustaceous, but usually corticate when foliose, with green algal cells. Perithecia simple, immersed or superficial, entire or dimidiate, with an apical pore. Ascus with 1-8, occasionally 16 spores. Paraphyses soon becoming mucilaginous and disappearing or more-or-less persistent. Spores simple, colourless or sometimes brown.

Paraphyses soon becoming mucilaginous and disappearing.

Spores vermiform and clavate at ends ... *Sarcopyrenia*

Spores \pm ellipsoid and not clavate at ends.

Thallus crustaceous ... *Verrucaria*

Thallus \pm foliose ... *Dermatocarpon*

Paraphyses persisting and usually branched.

Thallus crustaceous.

Hymenial algae present ... *Thelenidia*

Hymenial algae absent ... *Thrombium*

Thallus foliose ... *Anapyrenium*

Family MONOBLASTIACEAE

Thallus crustaceous or foliose with *Trentepohlia* or allied alga (*Phyllactidium* in *Haplopyrenula*). Perithecia simple, with an apical pore; ascus with 2-8 simple spores.

This family includes *Coccotrema* and *Monoblastia* from "Pyrenulaceae," *Haplopyrenula* from "Strigulaceae" and *Lepolichen*, the single representative of "Phyllopyreniaceae."

Thallus foliose ... *Lepolichen*

Thallus crustaceous.

Paraphyses branched and entangled.

Algal cells *Trentepohlia* ... *Monoblastia*

Algal cells *Phyllactidium* ... *Haplopyrenula*

Paraphyses simple and free ... *Coccotrema*

Family THELIDIACEAE

Thallus crustaceous or foliose, rarely fruticose, non-corticate when crustaceous, but usually corticate when foliose or fruticose. Algal cells green. Perithecia simple, immersed or superficial, entire or dimidiate, with an apical ostiole. Paraphyses soon becoming mucilaginous and disappearing, or more-or-less persistent. Spores transversely septate or muriform, colourless or dark.

Paraphyses soon becoming mucilaginous and disappearing.

Spores 1-3-septate (5-7 in *Normandina*).

Thallus crustaceous. Spores colourless ... *Thelidium*

Thallus foliose.

Spores 5-7-septate, elongate ... *Normandina*

Spores 1-3-septate, \pm ellipsoid.

Spores colourless ... *Placidiopsis*

Spores brown ... *Heterocarpon*

Thallus fruticose. Spores colourless ... *Nylanderella*

Spores muriform.

Hymenial algae absent.

Thallus crustaceous ... *Polyblastia*

Thallus more-or-less foliose ... *Agonimia*

Thallus more-or-less fruticose ... *Pyrenothamnium*

Hymenial algae present.

Thallus crustaceous ... *Staurothele*

Thallus more-or-less foliose ... *Endocarpon*

Paraphyses remaining. Hymenial algae absent.

Spores 1-septate and dark ... *Thelidiopsis*

Spores 3- or more-septate, colourless, fusiform.

Perithecia expanded above into a shield ... *Aspidopyrenium*

Perithecia immersed ... *Geisleria*

Spores acicular with many (14-19) septa ... *Gongylia*

Spores muriform (eventually).

Perithecia expanded above into a shield ... *Aspidothelium*

Perithecia immersed or free but not expanded.

Thallus crustaceous. Spores often

brownish ... *Microglæna*

Thallus \pm foliose. Spores colourless ... *Psoroglaena*

Family ARTHOPYRENIACEAE

Thallus crustaceous, non-corticate or rarely \pm corticate above, without rhizinae, with *Trentepohlia* or allied alga. Perithecia simple

or rarely grouped in a stroma, sessile or immersed, vertically placed with apical ostiole. Asci ovate or cylindrical, 1-8-spored. Paraphyses branched and more-or-less entangled, sometimes disappearing. Spores 1-many-septate or muriform, usually colourless but sometimes brown; cells cylindrical or cubical with thin walls.

Spores \pm ellipsoid (occasionally acicular), 1-many-septate, colourless.

Spores 1-septate, broad. Asci elongate. Paraphyses branched and entangled *Acrocordia*
 Spores 1-3-(5)-septate, longly-ellipsoid. Paraphyses often disappearing.

Perithecia not in stromata *Arthopyrenia*

Perithecia in stromata *Tomasellia*

Spores 3-9-septate, elongate or fusiform. Paraphyses branched and entangled.

Perithecia not in stromata.

Algal cells *Trentepohlia* *Pseudosagedia*

Algal cells *Phyllactidium* or *Heterothallus* *Raciborskiella*

Perithecia in stromata *Athrismidium*

Spores 1-many-septate, acicular. Paraphyses branched and entangled.

Perithecia simple *Leptorhaphis*

Perithecia in stromata *Celothelium*

Spores ovate or elongate-fusiform, brown, 1-3-(5)-septate.

Algal cells *Trentepohlia* *Microthelia*

Algal cells *Phyllactidium* *Microtheliopsis*

Spores muriform, colourless or brown.

Perithecia simple. Spores colourless *Polyblastiopsis*

Perithecia in stromata.

Spores colourless *Laurera*

Spores brown *Bottaria*

Family PORINACEAE

Thallus crustaceous, non-corticate, without rhizinae, with *Trentepohlia* or allied algal cells. Perithecia single (in known representatives), sessile or immersed, vertically placed and with apical ostiole. Paraphyses simple and free, usually persisting. Ascus usually elongate with 8 or fewer spores (in *Thelopsis*¹ many-spored). Spores 1-many-septate or muriform, usually colourless but occasionally brown; cells cylindrical or cubical with thin walls.

¹ See p 115

- Ascus with many (o)-1-3-septate spores ... *Thelopsis*¹
 Ascus with 8 or fewer spores.
 Spores 1-many-septate, colourless.
 Asci and paraphyses persisting. Spores elongate.
 Algal cells *Trentepohlia* *Porina*
 Alga *Phyllactidium* (or *Heterothallus*) Epiphyllous
 Perithecium naked.
 Thallus indefinite *Phylloporina*
 Thallus of definite spots with radiate
 margin *Strigula*
 Perithecium with stiff hairs on it ... *Trichothelium*
 Asci or paraphyses becoming \pm slimy. Spores many-septate,
 acicular.
 Alga *Trentepohlia*. Ascus becoming slimy *Belonia*
 Alga *Phycopeltis*. Paraphyses becoming
 slimy *Phylloblastia*
 Spores 1-many-septate, brown *Blastodesmia*
 Spores muriform, colourless.
 Algal cells *Trentepohlia* *Clathroporina*
 Algal cells *Phyllactidium* *Phyllobathelium*

Family PYRENULACEAE

Thallus crustaceous, non-corticate, or rarely \pm corticate above,
 without rhizinae, with *Trentepohlia* or allied alga. Perithecia simple
 or aggregate or in stromata, vertically placed and with apical ostiole.
 Ascus 1-8-spored. Paraphyses usually simple, free and persisting.
 Spores 1-many-septate to muriform, colourless or brown; cells
 lens-shaped, rounded or angular, with thick walls.

Paraphyses simple and free.

Spores 1-more-septate, often 3-septate and fusiform.

Algal cells *Trentepohlia*. Spores brown.

Perithecia in stromata *Melanotheca*

Perithecia not in stromata *Pyrenula*

Algal cells *Phycopeltis*. Spores colourless to
 brownish *Micropyrenula*

Spores muriform.

Perithecium naked. Spores dark *Anthracotheicum*

Perithecium beset with stiff hairs. Spores
 colourless *Stereochlamys*

¹ See p. 115.

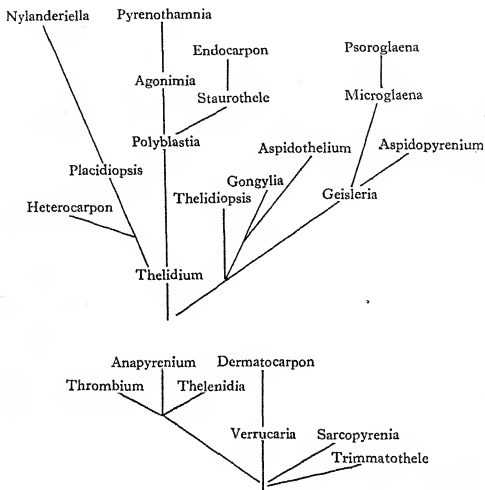
Paraphyses branched and \pm entangled. Spores 3-many-celled, colourless.

Perithecia simple or congregate but not in stromata

Pseudopyrenula

Perithecia in stromata

Trypethelium



Family ASTROTHELIACEAE

One of the important characters of this family is the more-or-less radiate arrangement of the perithecia. This is usually associated with other characters and may therefore be considered important, but otherwise does not deserve to have much taxonomic value given to it. Amongst the Graphidales the radiate arrangement is not considered to be generically important, or even, in some cases, specifically important. In both *Opegrapha* and *Graphis* there are some species, in which the apothecia are free from one another, and others in which they are radiately arranged. *Opegrapha vulgata* has a variety

*siderella*¹, with the apothecia in radio-stellate groups, whilst *Graphis scripta* and *G. elegans* also have forms or varieties in which the apothecia are stellately arranged.

In Astrotheliaceae the radiate grouping of the perithecia is associated with other characters. The perithecia are often oblique and united in stromata, and the elongated ostioles usually coalesce so as to form an elongated common canal. The association of these characters justify the formation of one or more groups. The varying nature of the spores and paraphyses in the genera listed under "Astrotheliaceae" does not indicate any single origin. The spore-cells may be either cylindrical, as in *Lithothelium*, or lens-shaped, as in *Astrothelium*, whilst the paraphyses are simple in the former and branched in the latter. In the allied family Paratheliaceae the perithecia are neither radiately arranged nor united in stromata, but are either oblique or open by elongated and oblique canals. Here again, an artificial grouping is indicated by the varying nature of the spores and paraphyses. There are three distinct origins for these two "families," the probable origins being from the lines which gave rise to *Pseudopyrenula*, *Polyblastiopsis* and *Porina* respectively. Accordingly the genera are distributed amongst three families: Astrotheliaceae (emended), Pleurotremaceae and Cryptotheliaceae. The emended family Astrotheliaceae is limited to four genera, two of which were placed by Zahlbruckner in it, whilst the other two were put in "Paratheliaceae."

The characters of the emended family are as follows:

Thallus crustaceous, non-corticate or somewhat corticate above, with *Trentepohlia*. Perithecia single or in stromata, sometimes radiately grouped, obliquely or vertically placed with ostioles oblique or lateral; ostioles elongated and sometimes coalescent into a common canal. Paraphyses branched and entangled or coherent. Spores 2-many-septate with \pm lens-shaped, or rounded cells, with thick walls.

Spores colourless.

Perithecia single	<i>Plagiotrema</i>
Perithecia radiate and in a stroma	<i>Astrothelium</i>

Spores brown.

Perithecia single	<i>Parathelium</i>
Perithecia \pm united or in a stroma	<i>Pyrenastrum</i>

¹ This may be given specific rank as *O. siderella* because of the short spermatia: in that case, *O. siderella* itself has a var. *subsiderella* in which the apothecia are not radio-stellate.

Family CRYPTOTHELIACEAE

Characters as in Astrotheliaceae except that the paraphyses are simple and free, or almost so, and that the spore-cells are more-or-less cylindrical. The two genera placed here are stromoid. *Lithothelium* has 3-septate and colourless spores whilst in *Cryptothelium* they are muriform and colourless.

Family MYCOPORACEAE

When the doubtful lichen *Asteroporum* is excluded, the characters and genera given by Zahlbruckner (36) pp. 92-3) can be accepted.

Family XANTHOPYRENIACEAE

The critical character is the presence of *Xanthocapsa* as the algal symbiont. *Xanthopyrenia*, the only known representative, has simple perithecia, 1-septate, colourless spores and a crustaceous homoiomerous thallus.

Family PYRENOTRICHACEAE

The critical character is that the byssaceous thallus has *Scytonema* algae. The family contains two genera which agree fairly well in the characters of the perithecia except that *Rhodothrix* has 1-septate and *Pyrenothrix* muriform spores.

Family MASTODIACEAE

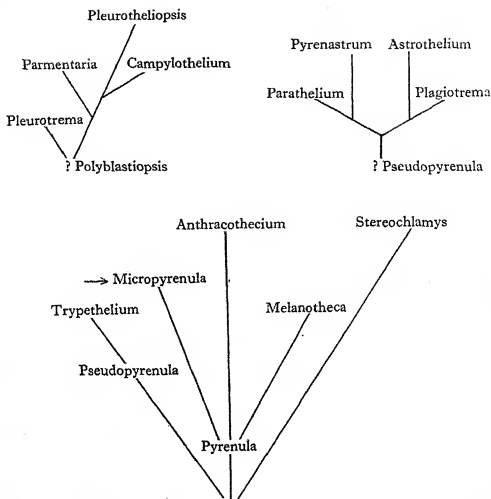
The critical character is the occurrence of *Prasiola* as the algal constituent. *Mastodia*, the only known representative, has simple perithecia, colourless and simple spores, whilst the thallus is homoiomerous.

Family PYRENIDIACEAE

This is admittedly a family of doubtful value and is only provisional. In it are placed most of the pyrenocarpous lichens having blue-green algal symbionts.

Pyrenidium has a brownish 3-septate spore, the structure of the supposed thallus is similar to one of the fruticulose *Leptogia*, and the perithecia may belong to a parasitic fungus. The other two corticate lichens placed in this group are *Cocciscia* and *Coriscium*, and the thecia of the latter are unknown. *Rhabdospora*, *Hassea*, *Placothelium* and *Eolichen* have the thallus non-corticate; the first two have simple spores whilst the latter has 1-septate spores and its lichen nature is not well established. *Placothelium* has *Dactylococcus* algae and the ascus contains many small spores¹. *Obryzum*, which is

¹ See p. 115.



placed in this family by Lorrain Smith, has no thallus, and is not admitted to be a lichen by most lichenologists. *Lophothelium*, which is doubtfully included by Zahlbruckner, has recently been re-examined by Lorrain Smith, who considers that the blue-green algae were merely associates, and that it is a fungus (*Discothecium*) parasitic on the squamules of *Stereocaulon condensatum* (32 a) p. 384.

Family CRYPTOTHECIACEAE

Characters and genera as given by Lorrain Smith (32 e).

Family THELOCARPACEAE

Thelocarpon was doubtfully placed by Reinke (30) in the family Acarosporaceae and Zahlbruckner has accepted that arrangement. Lorrain Smith rejects that interpretation and insists on placing it in a separate family (32 a) p. 377. The absence of a horizontal thallus may justify this separation but, apart from the thalline character,

there seems no reason why the crustaceous *Trimmatothele* should not be included in the same family. Other pyrenocarpous lichens with the ascus many-spored are *Placothelium*, *Thelopsis*, *Epigloea* and *Baeotitthis*. In *Placothelium* and *Thelopsis* the algal constituents are respectively *Dactylococcus* and *Trentepohlia*, whereas in *Thelocarpon* they are distinctly protococcoid. *Placothelium* has been put in the family Pyrenidiaceae, a family of doubtful value, and its affinities are uncertain. *Thelopsis* is included in Porinaceae, though its position there is questionable. A provisional arrangement of these two genera with *Thelocarpon* and *Trimmatothele* may be less artificial than the arrangement previously adopted. *Epigloea* is given a family of its own whilst *Baeotitthis* seems more nearly akin to Morioliaceae.

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THE PROTEIN METABOLISM OF THE GREEN PLANT

A REVIEW

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THE problem of the nitrogen metabolism of the plant may be defined by the fact that carbon dioxide, water and ammonium salts or nitrates are assimilated by the green plant and built up into a series of complex organic nitrogen compounds, which may be classified as the amino acids and their condensation products the proteins; chlorophyll; amides; cyanogenetic compounds; simple bases, including amines and betaines; purine bases and a special and complex group of bases, the alkaloids.

EARLIER WORK ON THE SOURCES OF NITROGEN

Gasometric experiments carried out by de Saussure in 1804 indicated that atmospheric nitrogen cannot be utilised by the higher plants; this suggestion was confirmed by Boussingault (*ib*) in 1860. In one classical experiment carried out by this investigator, three sunflower seeds were planted in purified sand; the first was treated with distilled water, the second with a solution of essential salts, exclusive of nitrogen compounds, and the third with a complete nutrient salt medium, containing potassium nitrate as a source of nitrogen; each plant was placed under a large bell jar, inverted over sulphuric acid to preclude the absorption of ammonia or the oxides of nitrogen in the atmosphere, and water and carbon dioxide were introduced through glass tubes, so that the bell jars were not removed throughout the course of the experiment. Eighty-six days after sowing, the following results were noted: the plant which had received potassium nitrate had developed in a completely normal manner, resembling one which had grown under favourable natural conditions, whereas the two which had been dependent on the supply of nitrogen present in the seeds were in a rudimentary state of development, being about one-seventh the height of the plant supplied with potassium nitrate.

Analyses yielded the following figures:

	Increase of dry weight in mg.	Increase of nitrogen in mg.
Seedling on sand with water	0.28	2.3
Seedling on sand with mineral salts	0.39	2.7
Seedling on sand with complete nutrient medium	21.11	116.7

The differences between the figures for the increase of nitrogen, 2.3 and 2.7, may be considered undoubtedly to be due to experimental error, and to the individual variation between two seeds.

This experiment is furthermore interesting in that it demonstrates the efficiency of the simple inorganic salt, potassium nitrate, as the sole source of nitrogen for a green plant. Previous to the work of Boussingault, it had been generally considered that ammonium compounds were of paramount importance in plant nutrition, this view being chiefly due to the influence of Liebig, who had first called attention to the significance of ammonia in rainfall to the growth of vegetation. The comparative values of nitrates and ammonium salts as sources of nitrogen will be discussed below.

Boussingault himself carried out estimations of the ammonia content of rain water and Barral in 1851 made similar analyses for nitric acid. Since that time careful experiments, carried out at Rothamsted by Lawes and Gilbert, have shown that the nitrogen carried down by rain and added to the soil in one year is approximately as follows:

2.4 lb. per acre as ammonia
1.0 " nitrates and nitrites
1.0 " nitrogen in organic combination
4.4 lb. total combined nitrogen (average value over five years)

Gilbert estimated that a crop of wheat from unmanured soil removed twenty pounds of nitrogen per acre, and that the amount of combined nitrogen received from the annual rainfall is approximately balanced by losses through drainage. In order that plant life may be maintained, it is evident that there must be other ways in which nitrogen is added to the soil in nature. It is now generally agreed that this is brought about by the action of saprophytic and symbiotic micro-organisms.

SYNTHESIS OF PROTEINS AND OTHER ORGANIC NITROGEN COMPOUNDS

It is natural that of all the syntheses of nitrogen compounds carried out by the plant, it is that of the proteins which has aroused the greatest interest. These substances are of particular importance to the biologist in that the living cells of each species of animal or plant contain proteins characteristic of that species only. The pro-

teins thus appear to provide the fundamental basis for structural individuality, the other essential constituents of the living cell, namely, the mineral salts, lipins and carbohydrates, being, up to our present knowledge, of more universally similar composition. The physical properties of proteins, moreover, apparently adapt them to the performance of those functions compatible with the activities of life, although the idea of a basic living substance "protoplasm" essentially of protein composition is now generally rejected in favour of a conception such as that propounded by Hopkins, namely, that life may be regarded as the manifestation of a dynamic equilibrium between the essential cell constituents, the colloidal nature of the protein providing an ideal reaction medium.

It has long been recognised that the animal organism is unable to carry out the primary synthesis of the majority of the structural units of protein, namely, the amino acids. The method by which the plant brings about these syntheses is as yet obscure, a fact which is hardly surprising if the uncertainty as to the actual structure of the protein molecule be considered.

At the present time, there are two main hypotheses as to the structure of the protein molecule. The classical researches of Emil Fischer led to the view that the natural proteins consist of about twenty amino acids, united to one another by peptide linkages. It is known from the recent work of Adair⁽³⁾ that the animal albumins have a molecular weight of about 68,000, and the animal globulins about 150,000, while the figure 17,000 has been given by this investigator as a provisional estimate for the molecular weight of the meta protein edestin hydrochloride. It is obvious, therefore, that permutations of the arrangements of the amino acids in molecules of such magnitude could account for the variations between the proteins of each species.

More recently, however, it has been considered that a structural arrangement as conceived of by Fischer will not account for the instability of proteins, and for such phenomena as denaturation and gelatinisation, and many workers, including Abderhalden and his colleagues⁽⁴⁾, have postulated that the proteins are polymers of complex cyclic units possibly of the nature of diketopiperazines, the amino acids formed on protein hydrolysis being artefacts caused by the decomposition of such cyclic systems. The only real evidence for such a view seems, however, to be that obtained by Brill⁽¹⁵⁾ in investigations of the Röntgen spectra of silk fibroin, which is not strictly comparable with typical albumins and globulins.

The most important evidence in favour of Fischer's view is the fact that peptides and amino acids are obtained from proteins by the action of naturally occurring enzymes, and furthermore that certain synthetic polypeptides can be hydrolysed by enzyme action. The work of Borsook and Wasteneys (14), moreover, indicates that a synthesis of protein from peptones may be brought about by the action of pepsin. The cyclic products obtained by Abderhalden from proteins, such as diprolylglycyloxyprolinanhydride, have so far proved to be resistant to enzyme action. The recent work of Hopkins indicates, moreover, that peptides are of an extraordinarily unstable nature, their activities being probably sufficient to explain any instability of the protein molecule. It is possible, however, as suggested by Bergmann (11), that the diamino and dicarboxylic acids are capable of more variation in their methods of combination than was originally suggested by Fischer.

According to the two hypotheses outlined above, two possible methods of synthesis of proteins by plants may be considered:

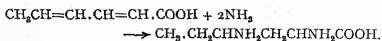
1. The classical theory of Fischer would suggest that the amino acids are synthesised separately, then condensed by a series of stages, the converse of hydrolytic processes, to form peptides, peptones, proteoses and proteins.

2. The recent polymerisation theory might indicate that the cyclic entities which become associated to form the protein molecule are laid down *en bloc*, according to a definite pattern unique to the cell concerned, from simple units such as ammonia, urea and carbonic acid, or aldehydes, ketones or carboxylic acids of low molecular weight.

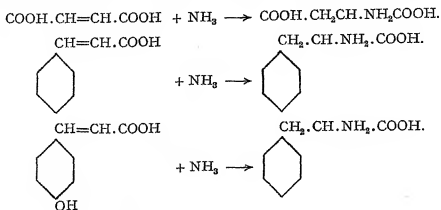
It is difficult to devise any test for either of these hypothetical methods of protein formation.

Many theories have been advanced as to possible methods of synthesis of amino acids. It has been stated above that ammonia is an important source of nitrogen for the green plant, the amino acids might therefore arise by amination of a corresponding series of non-nitrogenous acids. The carbon residues are generally considered to be derived from hexoses, or some active precursor or oxidation product of these substances.

It is known that in certain cases, ammonia may combine with unsaturated acids: diamino-caproic acid, for instance, has been obtained by the action of ammonia on sorbic acid:

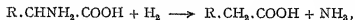


By analogy, aspartic acid, phenylalanine and tyrosine might arise from fumaric, cinnamic, and para-coumaric acids respectively:



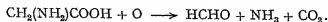
Fumaric acid is frequently found in plants; moreover the work of Quastel and his colleagues (60, 61) on bacteria has shown the equilibrium between fumaric and aspartic acids to be a possible biological phenomenon. Cinnamic acid and para-coumaric acid are probable plant constituents, but only few of the acids necessary for such processes have yet been isolated from plants.

Deamination is known to be brought about by living organisms; it is considered theoretically that any enzyme action may take place in either direction according to equilibrium conditions; the reversal of the process stated below may thus participate in amino acid synthesis:



that is, reduction of an amino acid, with formation of the corresponding fatty acid. Of these substances, isovaleric, succinic and glutaric acids are known to be widely distributed in plants, while acetic, propionic, isocaproic, and β -phenyl propionic acid and its parahydroxy derivatives are probably to be found.

That oxidative deamination can occur through the agency of certain catalysts present in plants was suggested by Chodat and Schweizer (23) and proved by the present writer (62) and by Happold and Raper (36). The reaction has been shown to take place as follows:



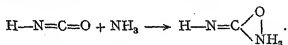
Decarboxylation, however, has not yet been shown to be a reversible process.

The work of Embden (28) and his colleagues has shown that if an isolated surviving liver be perfused with ammonium salts of certain hydroxy or ketonic acids, amino acids of corresponding constitution

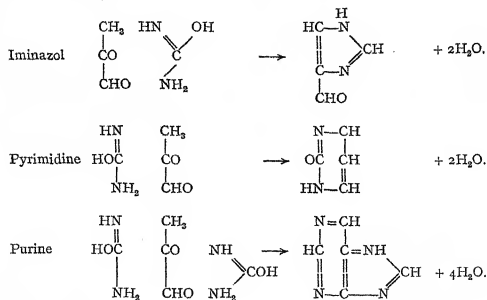
are obtained. Such experiments can naturally only be carried out on plants in a very indirect manner; attempts to prove the possibility of such syntheses by plants have been made by Smirnow (72).

It has been suggested by Kostytchew (43) that urea may be of importance in the synthesis of arginine, histidine and the purine bases. In recent years Fosse (29, 30), with the use of the xanthidrol reagent, has been able to isolate urea from many of the phanerogams, and it is considered by many that the low urea content or the apparent absence of urea in the higher plants is more to be attributed to rapid utilisation of this substance by the organism than to paucity of its production. The enzyme urease is known to be widely distributed in plants.

Urea can theoretically be derived as a condensation product of cyanic acid and ammonia:



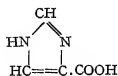
It might serve as the basis for certain heterocyclic rings in the following way:



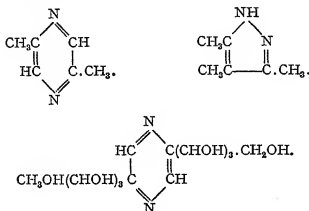
Methyl glyoxal is generally considered to be a probable stage in the biological oxidation or fermentation of carbohydrates. It must be emphasised, however, that such processes are purely hypothetical.

Certain reactions which have been obtained *in vitro* may be mentioned as being possibly related to the present problem. The work of Windaus and Ullrich (79) showed that the action of ammoniacal copper oxide on glucose easily brings about the formation of

β -iminazol carboxylic acid, this corresponding to the heterocyclic group of histidine:



Many workers have obtained evidence of the formation of cyclic compounds from ammonia and sugar (Dennstedt (26), Stoeck (74), Bamberger and Einhorn (8)). Substances of the following nature have been obtained in this way:



The suggestions as to the possible methods of synthesis of amino acids outlined above all involve the participation of ammonia. As stated previously, inorganic nitrates provide an important source of nitrogen for the green plant, and nitrates have been shown to be present in many plants (Borodin (12)). It is generally considered that nitrates undergo reduction to nitrites and ammonia in the plant before the nitrogen can be utilised for metabolic purposes. The process of the reduction has been studied by Warburg (77) in the case of the green alga *Chlorella*, and Godlewski and Nabokisch (31) have verified the production of nitrites by green plants in pure culture on media containing nitrates. In water-culture experiments weak solutions of nitrites are said to provide a suitable source of nitrogen for the higher plants, provided that there is an ample carbohydrate supply and that the reaction of the medium is carefully controlled (ref. Prianischnikow (59)).

A nitrate-reducing enzyme of the water-splitting type was detected in certain plants by Bach (6); this was shown to bring about the reduction of nitrates to nitrites in the presence of a suitable hydrogen acceptor, such as aldehyde. Anderson (4) has stated however

that this enzyme is not widely distributed, although in many of the cases tried it may have been undetectable by the methods used.

✓ If the utilisation of nitrate involves an obligatory reduction to ammonia, it seems anomalous that nitrates should be, on the whole, more valuable nitrogenous fertilisers than ammonium salts, as an inevitable expenditure of energy must be involved in their reduction by the plant. Such energy changes have been studied by Warburg (77) in the case of the assimilation of nitrates by *Chlorella*. In agriculture, nitrification is known to be highly beneficial to plant growth, whereas the presence of ammonia in unusual quantities is regarded as an indication of a poor soil.

The question of the relative values of nitrates and ammonium salts in plant nutrition has been studied by Prianschnikow and his pupils (57, 59). From evidence obtained from a long series of water-culture experiments, this worker concluded that two factors were of paramount importance in the production of organic nitrogen compounds from inorganic ammonium salts, namely, the amount of carbohydrate present, and the hydrogen-ion concentration of the medium, whether it be a nutrient solution or the soil.

Prianschnikow classified seedlings into three groups, according to their powers of synthesis of organic nitrogen compounds when growing in darkness on nutrient media containing ammonium salts.

I. Young grass seedlings, barley, oats and maize, and certain plants which have oil reserves in their seeds, such as *Cucurbita pepo*, showed an increase in size, dry weight and total nitrogen content, and could be shown to have synthesised asparagin when grown on such salts as ammonium chloride and ammonium sulphate. The acidity of the culture media was found to increase very definitely during the course of the experiments.

II. Seedlings of the Leguminosae which contained starch, such as *Pisum sativum*, *Vicia sativa* and *Vicia faba*, showed no increase of total nitrogen or of asparagin content when grown on ammonium salts of strong acids unless calcium carbonate or calcium sulphate were added to the media.

III. Seedlings of *Lupinus*, which contain small carbohydrate reserves, chiefly in the form of hemicelluloses, were unable to assimilate nitrogen from media containing ammonium chloride and ammonium sulphate even in the presence of calcium salts.

Etiolated seedlings of barley, namely, seedlings which had utilised almost all their available carbohydrate reserves, were unable to assimilate ammonia, but behaved analogously to *Lupinus*, whereas

lupin seedlings growing in the light and carrying out carbon assimilation, or growing in darkness on media containing glucose were able to take up ammonia and synthesise asparagin.

The explanation of these phenomena is as yet incomplete; the ammonium ion of the salt is evidently more rapidly absorbed by the root than the anion: the medium therefore becomes acid. The reason for the toleration of acidity by young grass seedlings is not clear, though apparently related to their high carbohydrate content. The method by which calcium salts enable certain seedlings of Leguminosae to utilise ammonium salts is also obscure. It is probable that the calcium ion has other effects besides that of neutralisation of the acid liberated in the medium; it has been stated that the seeds of many plants, chiefly the Leguminosae, do not contain enough calcium to enable the seedlings fully to utilise the carbohydrate reserves, thus, seedlings of *Phaseolus vulgaris*, germinating on distilled water, are said to die before the cotyledons are nearly depleted of their reserve starch, while they survive until the starch is exhausted if calcium salts be added to the medium. Calcium has been furthermore stated to be an important constituent of the cell wall, enabling it to withstand osmotic pressure. Prianischnikow (55), from evidence obtained from experiments on plants grown in the presence and absence of calcium sulphate, concluded that calcium increases the energy of growth, of respiration and of hydrolysis of the protein reserves of the seed, without changing the character of the metabolic processes or the translocation of soluble carbohydrate from the cotyledons to the axial organs. Smirnow (72) further studied the effect of the addition of calcium salts to media containing ammonium salts on which young barley seedlings were growing in darkness, and showed that, in the presence of calcium sulphate or calcium carbonate, a very rapid loss in the dry weight of the seedlings took place, while asparagin was not synthesised, the ammonia absorbed from the medium accumulating in the plant in the form of ammonium salts.

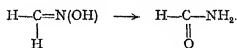
The growth of pea seedlings was found to be more rapid on ammonium salts of weak acids than on ammonium chloride and ammonium sulphate. Prianischnikow has stated that the actual excess of mineral acid is not noxious proportionately to the influence exerted on the reaction of the medium, but that the utilisation of the anion by the plant is also an important factor.

In experiments in which ammonium nitrate was used as the source of nitrogen in the culture media, the ammonium ion was shown

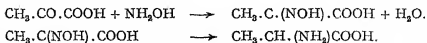
to be more rapidly absorbed by the plant than was the nitrate ion, the medium was therefore observed to become acid, so that mineral phosphates present could be brought into solution by means of the acid liberated.

Prianischnikow therefore considers that plants absorb ammonium salts preferentially to nitrates, as might be logically expected if nitrates must be reduced to ammonia before they can be utilised by the plant. He explains, however, that in natural conditions, nitrification is correlated with a well-aerated soil and the presence of bases, these circumstances being favourable for the growth of the higher plants, whereas ammonia predominates in soils which are insufficiently aerated or which contain organic acids or mineral complexes incompletely saturated by bases. Plants, moreover, have been found to be able to tolerate higher concentrations of nitrates than of ammonium salts, so that nitrification may be regarded as a safety valve by which plants are protected against an excessive ammonium content of the soil. These factors make the practical use of ammonium salts as fertilisers less conducive to plant growth than that of nitrates.

Several suggestions have been made, however, as to possible methods of utilisation of nitrates or nitrites without the formation of ammonia. Bach⁽⁵⁾ postulated the formation of hydroxylamine in the reduction of nitrites, and suggested a reaction between this substance and formaldehyde, yielding formaldoxime, which could then undergo a Beckmann transformation with the production of formamide:



It has further been suggested that α -isonitrosoketone could be formed by the interaction of nitrous acids and ketones, and that the oxime group can be easily reduced to an amino group. Another possibility is that hydroxylamine, acting on carbonyl groups would produce isonitroso groups:

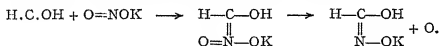


Baudisch and Meyer⁽⁶⁾ have considered that nitrate reduction in plants is a photochemical process, and postulated the reduction of nitrites to nitrosyl compounds, which might then combine with formaldehyde to yield formhydroxamic acid, which they regard as the first product of the assimilation of nitrogen. They were able to

show that the action of ultra-violet rays on nitrates in the presence of methyl alcohol leads to the formation of formhydroxamic acid, and claimed further that prolonged illumination caused the production of amino acids and cyclic compounds of the nature of pyrrol and pyridine.

More recently, Baly, Heilbron and Hudson (7) have also obtained the formation of formhydroxamic acid from solutions of potassium nitrite and carbonic acid exposed to ultra-violet light of wavelength $\lambda 290\mu$. They claim that activated formaldehyde, which they express as H.C.OH , that is, as possessing a divalent carbon atom, is formed in this process, and that it is the same as the first product of photosynthesis of the green plant. Aqueous solutions of formaldehyde exposed to ultra-violet light are thus stated to polymerise with the formation of reducing sugars, but if potassium nitrate be present, no sugars are formed.

The reaction is considered to proceed as follows:



The further interaction of formhydroxamic acid and active formaldehyde is said to produce complex nitrogenous substances, alleged to be similar to those found in the living plant, thus formhydroxamic acid is said to lose oxygen, and may be represented by

the formula $\text{H}-\underset{\text{NH}}{\underset{\parallel}{\text{C}}}-\text{OH}$, namely, a hydrate of prussic acid; this

substance might then react with active formaldehyde to give a labile ring compound $\text{HO}-\underset{\text{NH}}{\underset{\diagup \quad \diagdown}{\text{CH}}}-\text{CHOH}$ which, by internal rearrangement,

would give glycine, while, since the amino group is protected by internal salt formation, the median methyl group of glycine would react with formaldehyde to yield the homologues of glycine. The experimental evidence offered in support of this view is that such mixtures have been shown to give the ninhydrin reaction. It is stated, further, that methylamine and pyridine have been produced by the interaction of active formaldehyde and ammonia, whereas other experiments yielded traces of glyoxaline, a substance of alkaloidal nature, and a substance which reacted with diazobenzene sulphuric acid, and thus suggested to be histidine. The readiness with which these remarkable reactions take place is alleged to be due to the compounds involved being present in highly reactive phases, analogous to activated formaldehyde.

Whereas the evidence offered by Bach and Baudisch is not founded on biological experiments, and Baly's work has been the subject of much scathing criticism, there is no doubt that the question of the action of light on protein formation is of importance. It is perhaps natural to predict that photochemical processes are essential in such syntheses, considering that the presence of chlorophyll is perhaps the most striking chemical difference between the green plant and the animal, the latter being relatively deficient in powers of synthesis.

That light and the chlorophyll mechanism are not fundamentally essential for protein synthesis is proved by the growth of lower organisms on media containing only inorganic salts as sources of nitrogen. Abderhalden and Rona⁽²⁾ grew the mould *Aspergillus niger* on a culture medium containing potassium nitrate as the sole source of nitrogen, and cane sugar as that of carbon, and were able to isolate glycine, alanine, leucine, aspartic and glutamic acids from the mycelia; tyrosin and proline were, however, considered to be absent. Bayliss⁽¹⁰⁾, in a discussion of this work has remarked that this absence of cyclic derivatives is worthy of attention. It seemed to the present writer that the chlorophyll mechanism might be of particular significance in the production of aromatic and heterocyclic compounds. To test this assumption, *Aspergillus niger* was grown on a medium resembling that used by Abderhalden and Rona, and the mycelium obtained was autolysed for ten days at 37° in sterile conditions in an atmosphere of nitrogen. At the end of this period tryptophane and histidine were detected in the autolysate. Attempts to isolate the cyclic amino acids from an acid hydrolysate of *Aspergillus niger* are now in progress. It appears, therefore, that even cyclic amino acids can be formed by certain lower organisms in the absence of light or any chlorophyll system.

It would indeed be unwise to conclude, however, from evidence obtained from experiments on fungi and bacteria, that light is without direct action on the synthesis of amino acids or proteins in the higher plants. The indirect action of light is considered to be of significance, in that carbon compounds are presumably necessary to form the non-nitrogenous parts of the protein molecule.

It cannot be stated with any certainty that the primary formation of proteins or amino acids is restricted to any one region of the green plant, or whether all cells or all cambial cells are capable of synthesising protein *de novo*, that is from inorganic salts in the presence of carbohydrate. It is generally considered that all living cells have

the power of protein formation in that they can build up amino acids or peptides to form their own individual proteins. In the case of the animal cell, primary protein formation apparently cannot take place; the evidence on this question for all plant cells is however uncertain. If protein synthesis were considered always to take place by polymerisation of cyclic entities formed from simple molecules, according to the recent hypothesis outlined above, the distinction between primary and secondary production of protein in plants would probably cease to exist.

The green leaf is generally regarded as the organ of the plant which is pre-eminently concerned with synthetic processes, and there is some evidence, to be discussed below, which indicates that a primary synthesis of protein or amino acids takes place in the leaf, the necessary nitrogenous substances for secondary protein formation then being translocated to the growing point and the cambial region of the root and stem, where a rapid increase in protein is known to take place.

The experimental evidence relating to protein formation in the plant has chiefly been derived from a study of the green leaf, the germinating seedling and the ripening seed. The effect of light on protein synthesis has been studied by many workers, but the results obtained can hardly be regarded as conclusive.

Zaleski (81) carried out experiments on leaves of *Helianthus annuus*: these were cut off under water, and the laminae halved near the midrib. The halves containing the midrib were put with their petioles in a nutrient medium containing nitrates, with or without fructose, and kept for six to forty hours in darkness, while the other halves were immediately dried and analysed, as controls; at the end of the experiment the midribs were removed, and the residual leaf material dried and analysed. The results were calculated in terms of protein per square metre of leaf, and a small increase was noted. This work may be criticised on the grounds that the protein nitrogen was estimated according to the method of Stutzer. Kostytchew and Brilliant (44, 45, 46) have recently shown that amino acids may combine with sugars at low temperatures to give complexes precipitable by copper sulphate, so that the small increase in the amount of nitrogen precipitated by Stutzer's reagent in the above experiment (0.232 gm. per sq. m. of leaf) is not necessarily indicative of a real increase in protein nitrogen. Complete analyses of the soluble nitrogen compounds present are not given, so that it is impossible to decide, should a real increase of protein have been obtained,

whether or not this might have been the result of condensation of pre-formed amino-acids. In later experiments (Zaleski⁽⁸²⁾) a small but constant difference was found in the protein content of leaves kept under conditions similar to those stated above, in media with and without nitrates, this suggesting that nitrate has been absorbed and transformed into protein. The differences recorded are, however, very small.

A similar technique was used by Zaleski and Tutorski⁽⁸⁴⁾ in experiments from which they concluded that pea seedlings growing in darkness on synthetic media containing potassium nitrate, ammonium phosphate or ammonium aspartate, and various carbohydrates, showed, at the end of twenty days, an appreciable increase in protein content.

Similar criticisms as to technique can be applied to the work of Suzuki⁽⁷⁵⁾ and of Maliniak⁽⁴⁹⁾.

Goldberg⁽⁸³⁾ claimed further to have shown an increase in protein nitrogen in wheat embryos which had germinated in darkness. In such a case, however, a supply of amino acids or other translocatory organic nitrogen-containing substances were supplied by the endosperm, so that only condensation processes need have been involved.

The work of Stocklasa⁽⁷³⁾ seems to provide evidence that protein synthesis can take place in the absence of light. This investigator brought forward the view that the potassium ion may, in certain circumstances, be of importance in protein synthesis. His experiments on sugar-beet seedlings, grown in sterile media, yielded the following results: Twelve-day seedlings, allowed to develop in light in the absence of carbon dioxide, but on a complete nutrient medium containing sugar, appear to be able to form protein in the presence or absence of potassium: in darkness, the protein nitrogen increases only in the presence of potassium salts.

Stocklasa concluded therefore that protein synthesis is not a pure photochemical process, but that it can take place in darkness if carbohydrate and nitrate or ammonia and all essential mineral salts are present. Light is, however, considered to benefit these reactions as a supply of energy is thereby obtained. In the darkened plant it was suggested that potassium was able to accelerate the breakdown of carbohydrate by respiratory enzymes.

Stocklasa's experiments were unfortunately not entirely adequately controlled and it is difficult to decide from his data whether the increase of protein noted is really due to a synthesis from in-

organic nitrogen compounds or whether it might be brought about by condensation of amino acids present in the seedling.

Certain of the figures given by Smirnow for the nitrogen partition of maize seedlings which had been allowed to develop on ammonium aspartate in darkness indicate that, in these circumstances, a small but definite primary synthesis of protein had taken place. The matter is unfortunately not discussed by the author, but it is apparent that Prianischnikow and his pupils do not regard light as an essential factor in protein synthesis.

It seems therefore that the question as to whether light exercises a direct effect on the synthesis of protein or its precursors must as yet remain unanswered.

NITROGEN METABOLISM OF THE SEEDLING AND OF DEVELOPING SEEDS

The problem of the nitrogen metabolism of the seedling during germination has been very thoroughly investigated by Ernst Schulze and his pupils, who have made most valuable contributions to our knowledge of plant biochemistry and of protein chemistry, including the discovery and isolation of two amino acids, namely, phenyl alanine and arginine.

Investigation of the nitrogen metabolism of the germinating seed involves both the study of the breakdown of the reserve proteins of the endosperm or cotyledons and that of the regeneration of the degradation products to the proteins of the axial organs of the developing plant. The reserve proteins of seeds are chiefly of the class of globulins; small quantities of albumin are sometimes present, as in the pea, and certain Gramineae contain prolamines and glutelins.

A study of the etiolated seedling may be expected to yield information as to the intermediary products of protein metabolism, as protein regeneration is inhibited in the absence of light.

Unlike the animal organism, which excretes large quantities of nitrogen of endogenous and exogenous origin, the normal plant loses no trace of nitrogen throughout its life-cycle, except small quantities at leaf fall.

The most striking fact noted in the study of the chemistry of germinating seedlings is the abundant presence of asparagin; $\text{NH}_2\text{CO}\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\text{COOH}$ or of its homologue glutamin. Schulze and Castoro⁽⁸⁹⁾ showed that in etiolated seedlings of *Lupinus luteus* which were twenty-one days old, about half the total nitrogen of

the seedling and 73 per cent. of the degradation products of protein consisted of asparagin, only a very small proportion of which could have been accounted for by the aspartic acid present in the reserve protein. The accumulation of asparagin is especially notable in seedlings of the Leguminosae, but Schulze was able to detect the same phenomenon, to a lesser extent, in seedlings of *Papaver somniferum*, *Tropaeolum majus*, *Pinus silvestris* and *Picea excelsa*. The accumulation of glutamin was observed in other plants, notably certain of the Cruciferae, Caryophyllaceae, Euphorbiaceae and Chenopodiaceae. In certain plants, namely, *Helianthus annuus*, *Cucurbita pepo* and *Daucus carota*, both asparagin and glutamin were found in varying proportions, one amide in some individuals entirely replacing the other.

✓The accumulation of asparagin in such large quantity was explained by Pfeffer (54) as follows: The degradation of plant protein *in vivo* proceeds on quite different lines from the breakdown obtained by acid hydrolysis, asparagin being the chief primary product; this substance is to be regarded as the chief translocatory organic nitrogen compound. In germination asparagin is formed from the protein reserves and diffuses from the endosperm and cotyledons into the axial organs of the seedling, where it can bring about regeneration of protein in the presence of carbohydrate. In the etiolated seedling asparagin remains unchanged, serving as a nitrogen reserve.

Pfeffer's theory was supported by Borodin (13), who is generally said to have first shown that asparagin could accumulate in the vegetative organs of the plant, namely, in the leafy shoot, if light were withheld, although the fact was apparently known by Boussingault. Borodin realised that asparagin and carbohydrate could exist together in the seedling without the occurrence of protein synthesis, he therefore suggested a slight modification to the theory, namely, that glucose was the sole carbohydrate that could serve in protein regeneration from asparagin, so that if there were an intensive demand for glucose for other purposes, such as cell-wall formation, regeneration of protein did not take place.

Pfeffer's theory was of course propounded years before the work of Fischer and others had helped to elucidate the chemistry of the proteins. The view which he held of the rôle of asparagin as the chief translocatory organic nitrogen compound and the most important precursor of protein formation was maintained by Schulze and still finds its supporters (Chibnall (22)).

That asparagin and glutamin exist as such in the protein molecule

is extremely probable, as is shown by the work of Osborne (62). It is not to be expected that amides would appear as such in acid hydrolysates of protein, as the amide group in such circumstances would be saponified, leaving aspartic or glutaminic acid; the amount of ammonia obtained from an acid hydrolysis of a protein thus corresponds closely with that which would be yielded by amides of the dibasic amino acids which can thereby be isolated. Russian papers by Butkewitsch, Shemtschuschnikow and Petrow, cited by Kostytchew (43) state furthermore that in the autolysis of seedlings the amount of amide nitrogen increases, protein hydrolysis as catalysed by proteolytic enzymes proceeding without saponification of amides.

As stated before, however, the total amount of asparagin found in germinating seedlings can in no wise be accounted for by direct protein hydrolysis on the usual lines.

The classical researches of Schulze (63, 64) have shown that it is unnecessary to assume that the degradation of the protein reserves of seeds is carried out in a manner different from the normal action of proteolytic enzymes or acid hydrolysis. By a series of skilful analyses and isolations this worker proved that amino acids are the primary products of protein breakdown in germination and that the majority of the asparagin found is of secondary origin, having been formed chiefly at the expense of the monoamino acids, the concentration of which substances decreases during germination, although there is no regeneration of protein. Similar results were obtained by Prianschnikow (56) who emphasised the fact that the accumulation of asparagin continues after the protein breakdown has ceased, this being accompanied by a simultaneous reduction of the concentration of amino nitrogen present.

A characteristic analysis of the nitrogen partition in etiolated seedlings of *Lupinus angustifolius* at different periods of development yielded the following figures (Schulze (63)):

Table I. Per 100 parts ungerminated seeds, without testas

	Seeds	6-day seedlings	15-day seedlings	18-day seedlings
Protein nitrogen	6.14	3.19	1.49	1.51
Asparagin nitrogen	—	1.83	3.63	4.02
Nitrogen in phosphotungstic acid precipitates	0.42	0.49	0.45	0.43
Other soluble nitrogen (including monamino nitrogen)	0.05	1.10	1.04	0.65
	6.61	6.61	6.61	6.61

Schulze found, moreover, that the cotyledons, which contain the hydrolysing reserves of protein, contain but little asparagin in comparison with the amount of other non-protein nitrogen-containing compounds present, whereas the reverse is the case for the other parts of the plant. The following figures were given for *Lupinus luteus*:

Table II

Age of seedling (days)	Protein-free extract of cotyledons		Protein-free extract of remainder of plant	
	Asparagin nitrogen (%)	Residual soluble nitrogen (%)	Asparagin nitrogen (%)	Residual soluble nitrogen (%)
4	17.5	82.5	70.0	30.1
6	20.5	79.5	68.8	31.2
12	26.2	73.8	78.1	21.9

Similarly, from the endosperm of etiolated seedlings of *Ricinus communis*, with an average length of hypocotyl of 10-12 cm., tyrosin and arginin could be isolated, whereas neither asparagin nor glutamin could be identified. The residual portions of the plant yielded, however, considerable amounts of asparagin and only traces of arginine.

Qualitative analyses of Papilionaceae seedlings yielded the following results: after six days of germination, besides asparagin, leucine, tyrosin and the basic amino acids could be isolated, chiefly from the cotyledons. After two to three weeks of development leucine was found only in traces, tyrosin could not be detected, and arginine was only found in any appreciable quantity in *Lupinus luteus*. Considerable quantities of asparagin were obtained throughout.

Schulze laid emphasis on the statement that the accumulation of different amino acids in different species or even in the same species in varying circumstances does not involve special methods of protein breakdown in each case, but rather different rates of utilisation in metabolism of the acids liberated. Thus only a relatively small number of amino acids could be isolated even from very young seedlings, the others, for example alanine, which is indubitably present in the reserve proteins, could not be detected, and therefore may be presumed to undergo immediate secondary decomposition.

The only plant examined by Schulze in which he could not detect asparagin or glutamin was *Abies pectinata*, while *Picea excelsa* contained only small amounts of amide nitrogen. A considerable quantity of arginine could be obtained from both these plants; as mentioned above, this amino acid also accumulated to some extent in *Lupinus*

luteus. Schulze(65) however could not discover that arginine was formed synthetically during germination, or in any way acted as a means of storing nitrogen, although, like asparagin, this substance has been obtained from shoots of *Trifolium pratense* and *Medicago sativa* which have been kept with their stems in water for some days in a darkened room.

That the metabolism of diamino acids may differ from that of monoamino acids is indicated by the figures given in Table I, which show that whereas the monoamino nitrogen decreases as the asparagin nitrogen increases, the basic nitrogen remains fairly constant. The precipitates obtained with phosphotungstic acid may contain of course many other substances besides arginine, lysine and histidine (Vickery (76)), and as stated above, the arginine concentration has been shown to decrease in many plants during the course of germination. Certain probable metabolic changes undergone by arginine have been studied by Kiesel(39, 40) who demonstrated the presence of arginase in certain plants, and separated ornithine from autolysates of wheat seedlings and of *Vicia sativa*. Schulze regarded guanidin, which he isolated from seedlings of *Vicia sativa*, to be an oxidation product of arginine.

Most of the evidence regarding the secondary synthesis of protein, namely that presumably brought about by condensation of pre-formed organic nitrogen compounds, has been obtained by Schulze and his pupils (Schulze(67), Wassilieff (78)) from studies of the ripening seeds of Leguminosae. Further data have recently been published by Woodman and Engledow (80), for the nitrogen partition obtaining during the development of the wheat grain.

This process may be regarded as the converse of germination, in that it is a synthesis, in contrast to a hydrolysis, of reserve protein. During the ripening of the seeds of *Pisum*, a breakdown of protein has been shown to take place in the pod, while soluble nitrogen compounds pass from the pod to the seed, the pod thus serving to some extent as a reserve organ for the seeds. About one-half of the soluble nitrogen of the pods was found to consist of asparagin, while small quantities of various amino acids were identified, namely tryptophane, histidine and leucine and traces of arginine. Asparagin could not be obtained from the seeds, though glutamine was shown to be present; lysine, tyrosin and arginine were also isolated, the last named in such quantity that Schulze regarded a primary synthesis of this substance in the developing seed as a possibility.

The work of Schulze and Wassilieff indicated further that soluble

nitrogen compounds from the leaves of the plant might pass directly to the ripening seeds without necessarily first accumulating in the pods. An investigation of the soluble nitrogen content of the whole plant during the ripening of the seed showed that the amino acid concentration was extremely low, only leucine and certain of the bases being present in sufficient quantity for identification; asparagin however, could be isolated from all the plants examined, and often represented about 40 per cent. of the total soluble nitrogen of the plant.

The predominance of asparagin in the processes of germination and seed formation was regarded by Schulze as evidence that the acid amides are the materials par excellence for protein regeneration. In this opinion he is in close agreement with Pfeffer's hypothesis. The monoamino acids formed by hydrolysis of reserve proteins are considered to be unsuitable precursors of protein; they therefore undergo secondary decomposition, the process involved being probably that of oxidation. Similarly, in the case of the developing seed, the protein of the leaves and pods are chiefly translocated in the form of asparagin, which cannot be detected in the seed itself because it is there rapidly transformed into protein.

Additional evidence was brought forward by Schulze in support of his hypothesis as to the function of asparagin. Thus, an examination of normal green plants showed that such contained considerable quantities of asparagin, whereas the amino acid content was proportionately lower than that of etiolated seedlings at the same stage. He considered that this circumstance was due to the fact that conditions for the development of the normal plant being more favourable, the obligatory metabolic processes proceed more rapidly than is the case in the etiolated seedling.

In young normal plants, furthermore, the concentration of asparagin was found to be greater in the stems than in the leaves. The following figures are given for *Medicago sativa*, and there are many similar analyses:

Table III

Material	Weight (gram.)	Yield of asparagin (crystallised) (gram.)
Fresh leaves with petioles	170	0.4
Fresh leaves without petioles	200	0.1
Fresh stem	200	1.17
Fresh stem	390	1.50

The leaflets of etiolated seedlings of *Lupinus albus* were shown to yield 17.7 per cent. of their dry weight as asparagin, whereas in non-etiolated leaflets only 6.7 per cent. could be obtained, even when the petioles were included. Asparagin is therefore considered to undergo rapid metamorphosis to protein in the normal leaf.

The experiments of Hansteen⁽³⁵⁾ are also cited by Schulze as evidence that asparagin is the most suitable substance for protein regeneration. This worker studied protein formation in *Lemna minor* by a microchemical technique; this plant was shown to be able to accumulate starch if placed in solutions of glucose or cane sugar; if asparagin were added to the glucose solutions, no starch was found but protein reactions were obtained. The addition of asparagin to cane-sugar solutions did not lead to an apparent increase of protein, moreover addition of single amino acids to glucose did not have the same effect as asparagin. It is, of course, scarcely to be expected that one amino acid should lead to the formation of an entire protein molecule.

Kinoshita⁽⁴²⁾ is quoted, moreover, as having claimed to show that if etiolated seedlings of Soya are allowed to develop in sterile nutrient media containing 1 per cent. glycerine or methyl alcohol, the amount of asparagin originally present decreased, presumably having been utilised in protein synthesis.

The fact that Prianischnikow obtained a considerable accumulation of asparagin in etiolated seedlings, which had developed on nutrient media containing ammonium salts, might further be regarded as evidence that asparagin is of importance in the synthesis of organic nitrogen compounds. The evidence brought forward by Chibnall on this subject will be discussed below.

The hypothesis of Schulze might be considered to be in accordance with the most recent views as to the chemistry of the proteins, namely, that the amino acids as such are not actual constituents of the protein molecule, but are formed during hydrolytic processes. The work of Butkewitsch^(18, 19) is generally regarded as providing evidence that ammonia, liberated from the asparagin molecule by enzyme action, and not asparagin itself, is the direct precursor of protein. Asparagin and its homologue glutamin may therefore be regarded as substances particularly adapted to the transport of ammonia to regions where protein synthesis is taking place.

Prianischnikow is however opposed to the opinion that asparagin is to be regarded as a substance of paramount importance in protein synthesis, and has criticised much of the evidence brought forward

by Schulze in favour of Pfeffer's hypothesis. He considers that asparagin and carbohydrate cannot be said to bring about the regeneration of protein, as there is no direct evidence of the utilisation of asparagin in etiolated seedlings, even if carbohydrates be supplied. Asparagin formation moreover is known to be the most rapid in the early periods of germination, when the seed still contains adequate carbohydrate supplies; furthermore it is known that in certain tissues, such as the potato tuber, asparagin and glucose may exist side by side in appreciable concentrations, although no protein formation can be detected (Seliwanoff (71)). The experiments of Hansteen are considered to show only that certain organic nitrogen compounds inhibit starch formation.

Prianischnikow further lays emphasis on the results of experiments made by himself, and by Schulze, on seedlings which, after several days of etiolation, were placed in the light, the nitrogen partition being determined after some days of carbon assimilation had caused the onset of protein regeneration. A typical example of the result of such an experiment is given below, the material being seedlings which had been grown in sand and supplied with a medium containing no nitrogen.

Table IV

	Percentage of total nitrogen	
	Seedlings which had been kept for 10 days in darkness	Seedlings kept for 10 days in darkness then exposed to light for 3 weeks
Protein nitrogen	25.2	33.2
Asparagin nitrogen	34.2	42.0
Residual soluble nitrogen including amino acids	40.6	24.8

Prianischnikow considers therefore that protein is regenerated from the amino acids rather than from asparagin, as the concentration of the latter is shown to have increased.

Schulze, however, considers that this argument is not valid, as, in the condition obtaining in the above experiments, synthetic processes may not have entirely gained ascendancy over degradation processes, so that the increase of asparagin may be due to further secondary changes of amino acids which mask the effect of the utilisation of asparagin in protein formation.

Schulze (64) further emphasises the fact that it is not admissible to conclude that a substance which accumulates in a living organism is of little metabolic importance, or that it is utilised with difficulty.

He cites the analogy between the presence of asparagin and that of cane sugar in the developing lupin seedling. The quantity of cane sugar increases considerably during the course of germination, and yet it can in no wise be regarded as an unutilisable substance. It must be admitted, however, that certain of Schulze's arguments in favour of the utilisation of asparagin rather than that of amino acids, for protein synthesis, are based on reasoning analogous to that which he thus condemned.

Experiments of Zaleski and Shatkin (83) on *Allium cepa* seem to support the opinion expressed by Prianischnikow, namely that protein is regenerated from amino acids. If the bulbs of *Allium* were allowed to germinate over distilled water, an increase of protein nitrogen was observed. A study of the nitrogen partition showed that such an increase is accompanied by a decrease in concentration of amino nitrogen and not of amide nitrogen. In these experiments, amino nitrogen was estimated by Van Slyke's method.

Table V. Bulbs had germinated for 25 days.
Figures calculated in terms of total nitrogen

	Control (%)	Germinated bulbs (%)
Protein nitrogen	44.4	63.4
Peptone nitrogen	11.0	9.0
Ammonia nitrogen	4.5	3.0
Amide nitrogen	11.4	10.7
Basic nitrogen	3.5	2.7
Monoamino nitrogen	26.0	10.0

Prianischnikow therefore prefers to support the theory of Bous-singault rather than that of Pfeffer, as modified by Schulze. In a paper published in 1895 (Prianischnikow (55)) he quotes the following words of Boussingault, and his views at the present day are apparently unaltered:

L'animal de l'organisation la plus simple n'émet pas seulement, en respirant, de la chaleur, de l'eau, de l'acide carbonique; une partie de l'albumine qu'il consomme est modifiée par la combustion respiratoire en un composé azoté cristallin, l'urée, que l'on rencontre dans les excréments. Dans la combustion respiratoire, d'une plante vivant à l'obscurité une semblable modification de l'albumine ne pouvait être aussi manifeste, par la raison que les végétaux sont dépourvus d'organes excréteurs; mais dans les sucs, remplissant les cellules, on trouve un principe immédiat cristallin, l'asparagin, qui est un amide comme l'urée, en se transformant aussi facilement en aspartate d'ammoniaque que l'urée se transforme en carbonate d'ammoniaque.

Une graine qui germe, un végétal vivant dans un lieu obscur,

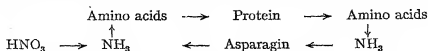
élaborent de l'asparagine. Une plante produit ce principe, même à lumière, dans les premières phases de la vie tant que domine la force éliminatrice, tant qu'elle laisse brûler plus de carbone qu'elle ne révivifie d'acide carbonique. D'ailleurs, dans le jeune âge, cette plante possède plus de racines placées à l'obscurité que de feuilles exposées à la lumière. Aussitôt que, par l'abondance des feuilles, la force réductrice vient à dominer la force éliminatrice, lorsque, par exemple, la plante est sur le point de fleurir, on ne rencontre plus d'asparagine, si ce n'est dans les racines très développées.

Dans une plante venue à l'obscurité, l'asparagine s'accumule par ce qu'elle n'est pas modifiée par l'action de la lumière. On la trouve dans les feuilles, dans les tiges, et dans les racines; c'est du moins que j'ai reconnu pour le maïs, le haricot, les pois, le trèfle.

Although Boussingault's statement as to the complete disappearance of asparagin in the normal mature plant is apparently exaggerated, there seems at the present time to be no real evidence that his general conception is erroneous, except perhaps the facts brought forward by Schulze as to the high asparagin content of normal green seedlings of certain Leguminosae; it must be considered however, that the carbohydrate metabolism of such seedlings has not been satisfactorily investigated.

Asparagin, according to Prianischnikow, may then be regarded as an innocuous means of storage of ammonia, the latter being injurious to the plant if present in quantity (Butkewitsch (18)). As such, the function of asparagin is analogous to that of urea in the animal, but, unlike urea, it is not excreted, but can be utilised for protein formation in favourable circumstances. Prianischnikow (58) draws an interesting parallel between the behaviour of lupin seedlings developing on ammonium salts of strong mineral acids and that of an isolated surviving mammalian liver on perfusion with these substances. Lupin seedlings, as stated above, are unable to absorb nitrogen from solutions of ammonium chloride and ammonium sulphate in the absence of carbohydrate, but, although their total nitrogen content remains unchanged in such circumstances, analysis of the nitrogen partition reveals an increase in the ammonia content, this ammonia having apparently been liberated from organic combination, namely, from asparagin. Similarly, perfusion of a surviving liver with ammonium chloride or ammonium sulphate is said to cause a decrease of urea production, more ammonia being obtained from the perfusion fluid than could have resulted from the salt added. Urea formation having been inhibited, ammonia of endogenous origin appears as such.

The following scheme for protein synthesis in the plant would appear to be in accordance with the views expressed by Prianischnikow:



The chemistry of asparagin production will be discussed below.

NITROGEN METABOLISM OF THE GREEN LEAF

The nitrogen metabolism of the green leaf presents a more complex problem than that of the germinating seedling or the ripening seed, as in the leaf, presumably, primary synthesis of amino acids or of protein takes place concurrently with protein breakdown and translocation of soluble nitrogen compounds to other parts of the plant.

Of recent years, experiments on the nitrogen metabolism of the leaves of *Phaseolus vulgaris* have been carried out by Chibnall (21, 22). This author has confirmed the results of Borodin (13), Schulze and Bosshard (68) and Butkewitsch (17) as to the increase of asparagin in starved leaves, namely, leaves placed for some days in darkness or diffused daylight with their petioles in water. Characteristic figures for the nitrogen partition in such experiments are given below:

Table VI

No. of days in water	In % of total leaf nitrogen				In % of total non-protein nitrogen		
	Total soluble nitrogen	Ammonia nitrogen	Amide nitrogen (Sachsse)	Amino nitrogen	Ammonia nitrogen	Amide nitrogen	Amino nitrogen
0	16.5	0.34	0.89	6.81	2.00	5.98	41.30
5	35.14	0.57	5.85	15.82	1.62	16.65	45.00

The above results show that in starved leaves, in terms of water-soluble nitrogen, the most striking increase is that of the amide nitrogen.

The seasonal and diurnal variations in the chemical composition of the leaves of *Acer negundo* have been investigated by B. Schulze and Schütz (70). The results obtained by these workers indicated a reduction in the total nitrogen content of the leaves at night, correlated with a decrease in protein nitrogen, during the period of physiological activity of the trees. The non-protein nitrogen content was shown to remain practically unaltered at night, which suggested that nitrogen has been translocated to other parts of the plant.

Chibnall⁽²²⁾ has repeated the experiments of Schulze and Schütz concerning nitrogen, using as material the leaves of *Phaseolus vulgaris*. Leaves examined in the month of August showed an average decrease of 1.8 per cent. of protein nitrogen at night, whereas the non-protein nitrogen appeared to decrease by about 9 per cent. The percentages of ammonia and of amide nitrogen remained practically unchanged.

It is unfortunate that neither B. Schulze and Schütz nor Chibnall gave any criticism of the work of Kosutany⁽⁴⁷⁾ who claimed to have shown that an increase of protein nitrogen took place at night in the case of *Riparia sawage*. The technique used by this worker does not appear to have been very reliable, but his results were accepted by E. Schulze⁽⁶⁴⁾.

Chibnall has concluded that there is a continuous decomposition of protein in the normal leaf, by day and by night, accompanied by translocation of soluble products to other parts of the plant. This effect is masked by day because the rate of protein synthesis is greater than that of degradation. He has deduced, from the experiments on starved leaves, that the chief translocatory product is asparagin, into which the protein is gradually converted, with the intermediate formation of amino acids. He is thus in agreement with the views of E. Schulze⁽⁶⁶⁾.

This deduction might be criticised on the grounds that asparagin, according to the opinion of Boussingault and Prianischnikow, would tend to increase during starvation and ensuing protein degradation merely on account of its properties as a convenient innocuous storage product. An accumulation of amino acids, on account of the probable specific dynamic action on metabolism of these substances, might well be regarded as an improbable occurrence, whereas they might be the usual translocatory products concerned with protein formation, their concentration in normal circumstances never exceeding a normal equilibrium value.

It may be mentioned that Chibnall's figures indicate that throughout the life of the leaf, including the period when pods are forming on the plant, the concentration of total amino nitrogen is considerably greater than that of asparagin nitrogen. These results are contrary to those obtained by Schulze for *Vicia sativa* and by Wassilieff for *Lupinus albus*. It is obvious however that very different results may be given by the use of the Van Slyke technique, employed by Chibnall, and by attempted isolations of amino acids as carried out by Schulze.

From the work of E. Schulze and his colleagues, and the results recently obtained by Chibnall, it might be concluded that, in the leaf, primary formation of protein may take place from ammonium salts, brought there from the soil by the transpiration current or obtained from nitrates by reduction, and from carbon compounds formed during photosynthesis, the ensuing structure of the protein molecule, or of the fundamental group which thereafter undergoes polymerisation, being governed by fields of force at some active centre. A continuous concurrent protein breakdown takes place, presumably through the agency of proteolytic enzymes, the amino acids thus formed undergoing deamination, while the ammonia liberated combines to form asparagin, which is then translocated to the growing point or to the cambial regions of the root and shoot, where ammonia is again liberated and protein formation again takes place by the same mechanism as that of the primary synthesis in the leaves. According to this view, it would logically follow that ammonium salts might proceed directly from the soil to the growing point, and there take part in protein synthesis together with carbonic acid or the products derived from translocatory carbohydrates. Furthermore it would seem, as suggested by Prianischnikow's experiments with seedlings growing on ammonium salts, that in the leaves asparagin might be formed *de novo*, and be used directly as a translocatory product.

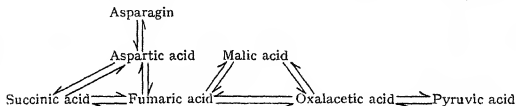
The alternative view, as stated above, is that synthesis of the individual amino acids takes place, presumably in the leaf, as being the organ of the plant life chiefly adapted for primary synthesis, but possibly also in other green tissues. The amino acids may either be built up into leaf protein, or translocated as such or as peptides to other growing parts of the plant where they are condensed to form proteins suitable to the tissue concerned, by the reversed action of the proteolytic enzymes.

In favour of this alternative, it may be said that fewer purely conjectural processes are involved. After primary formation of the amino acids has been achieved, in the photosynthetic organs where highly reactive molecules might be expected to occur, the further stages of protein formation could be explained by the activities of enzymes which are well known to be present (Kostytchew⁽⁴³⁾). The first hypothesis entails processes which are as yet beyond the range of experimental investigation; protein formation, moreover, would seem to proceed throughout on lines fundamentally different from those followed by protein degradation. It might be argued, however,

Amination of the succinic acid produced would then yield aspartic acid. Ammonium aspartate could then give rise to asparagin.

It is more generally considered that the amino acids undergo deamination, and that the carbon chain of asparagin is formed from the products of carbohydrate metabolism, the synthesis thus resembling that postulated by Prianischnikow in his experiments on primary asparagin formation in etiolated seedlings grown on media containing ammonium salts.

The following scheme might be suggested for asparagin formation:



Such reactions have been shown to take place in the presence of resting bacteria (Quastel (60, 61)). Succinic acid and malic acid are known to be present in many plants, and are presumably formed by carbohydrate metabolism.

In the case of bacteria, however, a similar equilibrium for glutamic acid and glutamin cannot be detected, and although the work of Moyle (50, 51) has shown that succinic acid is formed from glutamic acid in the presence of muscle, a reversal of this reaction, which involves decarboxylation, is not yet known to take place.

Only one case of an accumulation of aspartic acid has been recorded. In an investigation of the nitrogenous constituents of the rye plant during ripening of the grain, Kiesel (41) was unable to detect the presence of asparagin, whereas at one stage of ripening, aspartic acid was isolated in considerable quantity. It is not apparent, in this case, that the aspartic acid bore any definite relation to the storage or translocation of ammonia.

There is much contradictory evidence as to the existence of enzymes which are capable of liberating ammonia from asparagin (Clementi (24)). A deamidation of asparagin by plant extracts and autolysates was reported by Kiesel, and more recently Grover and Chibnall (33) have isolated aspartic acid from *L*-asparagin which had been incubated with a preparation made from rootlets of germinating barley, and which was shown further to liberate ammonia from α -glutamin and from urea, and to hydrolyse glycyl-glycine. Dernby (27) and Grover and Chibnall have considered that the

hydrolysis of asparagin is to be attributed to the action of a peptidase, asparagin being regarded as a dipeptide.

It may be surmised, from certain observations of Schulze⁽²³⁾ that the oxidation of amino acids, with subsequent formation of asparagin may take place in the germinating seedling owing to a relative deficiency in the carbohydrate supply. It was shown that the loss of reserve protein in a seedling was relatively greater if the seed contained only a small initial supply of non-nitrogenous reserve.

The following figures are given for etiolated seedlings after fifteen days' development:

Table VII

	% of original protein which had been hydrolysed	% non-protein reserves of original seed
<i>Lupinus luteus</i>	80.4	18 (carbohydrate)
<i>Lupinus angustifolius</i>	75.7	39 carbohydrate, 7.5 % fat
<i>Cucurbita pepo</i>	27.9	52 fat
<i>Zea mays</i>	27.4	Rich in starch

The rate of protein degradation and of asparagin formation is more rapid at the beginning of germination, when the seedling has apparently an adequate amount of carbohydrate; the form in which much of the carbohydrate is present may however be unavailable for respiratory purposes. This state of affairs was suggested by Schulze's experiments with germinating lupins, in which he showed that the soluble carbohydrate present in the seed, which he called lupeose, gradually disappeared and was replaced by cane sugar.

The accumulation of asparagin in the starved leaf might similarly be considered to occur on account of carbohydrate deficiency. The experiments of Deleano⁽²⁵⁾ are of interest in this connection. This investigator made a comparative study of the carbohydrate and nitrogen metabolism of leaves of *Vitis vinifera*: the leaves were halved and in one series of half leaves, estimations of carbohydrate and nitrogen content were made, while in the other half leaves the respiration was measured for definite periods, analyses being subsequently made as for the controls. The results showed that at the end of one hundred hours, the cane sugar and most of the starch had disappeared, and the concentration of reducing sugar had begun to fall, whereas, in the case of the nitrogen compounds, protein breakdown and an increase of non-coagulable nitrogen products apparently began concurrently with the exhaustion of reserve carbohydrates and the fall in concentration of reducing sugars. More carbon dioxide was shown to be evolved than could have been yielded theoretically

by the amount of carbohydrate and organic acids estimated to be present, the divergence between observed and calculated amounts beginning shortly after the one hundred hour period.

It is perhaps unorthodox to compare the state of the germinating seedling with that of the starved leaf, but it is clear that the mobilisation of carbohydrate and protein must be rapid in endospermic seeds, as the cotyledons must cease to form contact with the endospermic tissue before they can be withdrawn from the testa.

EMBRYONIC TISSUE CULTURE

One further interesting line of attack on the hitherto insoluble problem of the protein metabolism of plants is that of embryonic tissue culture.

Preliminary work of this nature was carried out by Hannig (34) who cultivated various Cruciferae embryos which had been removed from the seed. It is noteworthy that this worker was unable to observe an increase of protein content in embryos which had developed on a tryptic digest, whereas he claimed to have detected a synthesis of protein if the culture medium contained peptones and sugar. Somewhat similar results have recently been obtained by Carrell and Barker (20) in experiments on the culture of animal embryonic tissue. It must be stated however that the presence of toxic bases in the digests used in these experiments was not excluded.

The results of Holmes and Watchorn (37) obtained from skilful analyses of the nitrogen partition of embryonic mammalian tissues are difficult to explain in accordance with the orthodox views on nitrogen metabolism in the animal. It seems possible that the state of uncertainty which prevails as to the course of protein formation and its accompanying phenomena in the plant is more salutary than the state of quasi-security which has prevailed for the last decade as to nitrogen metabolism in the animal.

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THE STRUCTURE OF FASCIATED PLANTS OF *CAMPANULA CARPATICA* JACQ.

BY W. C. WORSDELL, A.L.S.

(With Plate IV and 8 figures in the text)

HISTORICAL

"RING-FASCIATION" has been observed and described by various authors in widely different plants. Although somewhat infrequent, it is by no means a rare phenomenon.

Reichardt and Michaelis describe remarkable and complicated cases of it in the scape of the dandelion (*Leontodon Taraxacum*). Conard observed the phenomenon in *Convolvulus Batatas*, Schoute in certain palms. Compton and also I myself have investigated it in *Pisum sativum*. The former has given us a detailed account of its occurrence in that plant, and I agree with most of his conclusions thereon. L. A. Boodle found it in *Althaea rosea*.

I have also observed it in connection with the curious "witches' broom"—or gall-formation on the branches of *Salix fragilis* and other willows.

In fact, the phenomenon is of very wide occurrence, and there is little advantage in citing every single case which has ever been noticed.

INTRODUCTION

The following pages embody the results of the investigation of the internal structure of the fasciated stems of certain individuals of *Campanula carpatica*¹ which were grown, for the purpose of genetic studies, in the grounds of the John Innes Horticultural Institution at Merton. The work was undertaken at the request of the Director, Sir Daniel Hall, and especially of Miss C. Pellew, who has undertaken breeding experiments with these plants.

I here insert an important account by Miss C. Pellew with regard to the genetic origin of the plants:

The fasciation here described in *Campanula carpatica* was first observed among the descendants of crosses between a wild strain and a horticultural form known as *pelviformis*. Genetical experi-

¹ They are all the products of crosses between various strains.

Structure of Fasciated Plants of *Campanula carpatica* 151

ments on *pelviformis* and other garden forms have been carried on for many years (*Journ. Genetics*, 1917, 6, 4), but no sign of fasciation had been observed prior to the introduction of the wild plants, in 1921. They were sent to me through the kindness of Professor Dr R. von Wettstein, and had been collected on the Crepătura at Zărnești. The plants sent had been numbered 8644, and I should be interested to know if, at the Cluj Botanical Garden, fasciated plants similar to mine have come from this original stock.

The fasciated type of growth is recessive to the normal, and the wild plants were a mixture of homozygous and heterozygous normals, as is shown by the experiments recorded below. It should be noted that *C. carpatica* is self-sterile.

A. Normal \times normal.

(1) Horticultural var. (Plant No. 7) \times wild.

30 plants normal.

One of these plants crossed back to wild.

12 plants normal.

3 plants fasciated.

(2) Wild \times wild.

(a) 10 plants normal.

(b) 21 plants normal.

15 plants fasciated.

(These plants have not yet all flowered, and the numbers given may not represent the real ratio. Assuming that in (b) both parents are heterozygous, the expected ratio is 3 normal : 1 fasciated.)

B. Normal \times fasciated, and reciprocal.

(1) The normal parent a horticultural variety.

56 plants normal.

(2) The normal parent a wild plant.

83 normal plants.

83 fasciated plants.

C. Fasciated \times fasciated.

60 plants fasciated.

Of the plants mentioned or examined by Mr Worsdell Nos. 27¹/25 and 29¹/25 were from the backcross (in A (1)); 56/26 and 58/26 from fasciated plants intercrossed (in C), and 123/26 and 125/26 from normal \times fasciated (in B (2)).

The stems of various strains indicated in the description by specific numbering and lettering, were investigated, and not all of them gave the same results. In some the peculiar structure described below is completely absent, the structure in these being precisely the same as in the normal, non-fasciated plants.

Externally, the fasciated stem has, in its lowest part, a normal, cylindrical contour. Higher up, it gradually increases in diameter, at first in one plane only, until a forking occurs; the stronger of the two resulting branches then thickens in a plane at right angles to the thickening of the primary stem, and at length also forks. A succession of forkings thus occurs in a more or less irregular manner right up the stem (Pl. IV, photo. 2).

As a rule, this robust, fasciated stem represents, or replaces, the very numerous branches into which the stem at once (quite close to the base) becomes resolved in *normal* plants of this species (Photo. 1). In some cases, however, there occur a few of these basal lateral branches in the fasciated plants, and two or three of these may be very strongly banded, while others are quite normal (Photo. 2).

"Ring-fasciation" only occurs in the thick, robust, fasciated stems, but by no means invariably in these, for some of the robust stems are entirely devoid of it. Its occurrence in these crossed plants is very sporadic.

The anatomical structure, known as ring-fasciation, described below, represents the expression, in the internal tissues of the stem, of the division by means of forking which occurs externally. But, as stated above, it does not occur in all the fasciated stems. For example, in a fasciated plant which was numbered 125⁸ the ring-fasciation was very markedly developed. Yet in another plant of the same brood, 125¹⁰, which was just as typically and strongly fasciated, there was no trace of any abnormal structure, which resembled in all respects that of ordinary, non-fasciated plants. In such stems of the 125¹⁰ type the forking takes place by simple invagination of the tissues on either side, without the formation of independent and (at first) isolated structures in the pith.

ORIGINAL OBSERVATIONS

Plant No. 123 G

Plants bearing the number 123 are the result of a cross between 27¹, a fasciated plant, and 8644 a normal "wild" plant. Of this brood 123 A, B, G, M, N are all fasciated, while 123 C, D, E, F are all normal.

I will now describe the ring-fasciation as it occurred in 123 G, and as observed in a series of transverse sections of the stem.

About an inch or rather less from the stem base, and in that region which had not yet begun to assume the fasciated condition,

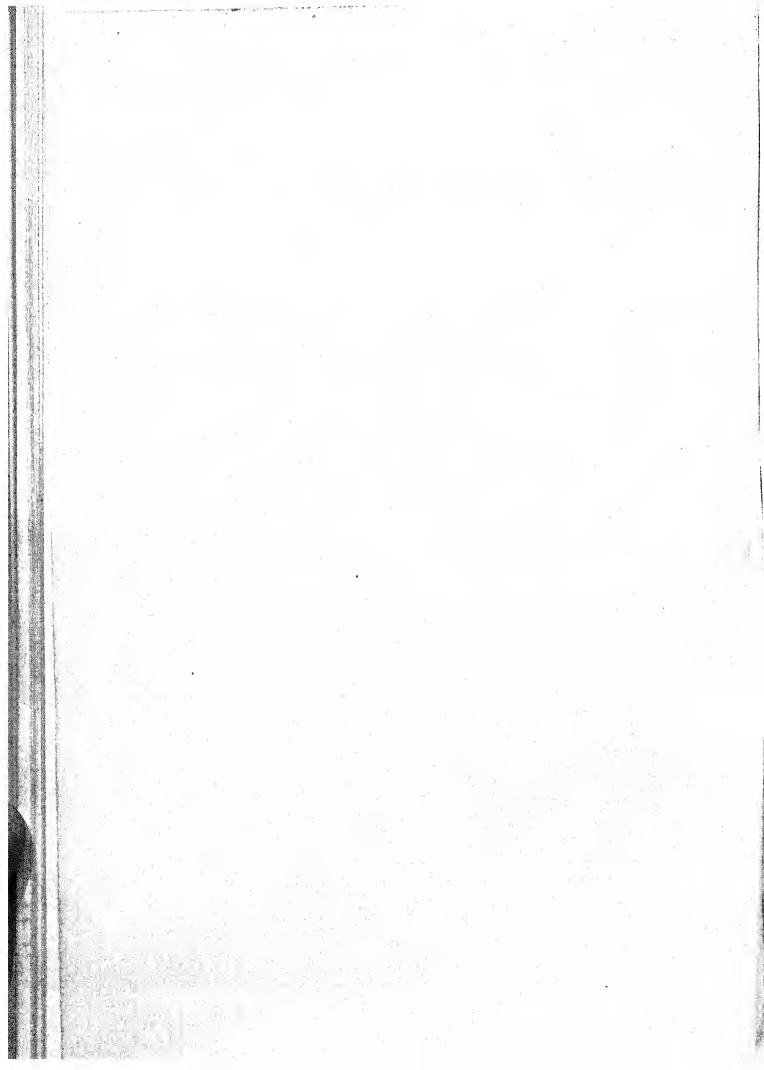
Photo. 1.



Photo. 1. *Campanula carpatica*. Normally branched stem of plant No. 124⁷.



Photo. 2. *Campanula carpatica*. Partially fasciated stem of plant No. 124¹³.
(The anatomy of this plant was not investigated.)



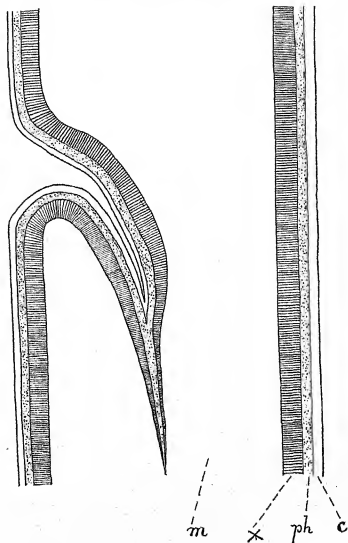
a minute strand arises *de novo* in the centre of the pith, consisting of very small elements with denser contents than those of the pith cells, but not yet differentiated into xylem and phloem. Two or three internodes (these being here each only a few millimetres long) higher up some tracheides develop and occupy the centre of the strand. But a little before this stage there arises all around the future vascular strand two oval rings of cambium, one on each side of the central strand, which form rows of ground-tissue cells, containing dense contents, both in the outward and inward direction, giving rise to an irregular zone of crushed pith cells as a result of the pressure exerted. At a slightly higher level these two cambium rings unite together all around the central vascular strand, forming thus a complete cylinder possessing both an inner and an outer ring of cambium. At a very slightly higher level the whole of this structure completely dies out, leaving the vascular strand in possession of the field.

I am unable to explain the presence of the above structure except on the supposition that it represents the vestige of a ring-fasciation which occurred in full development in some previous generation.

The central vascular strand increases in size, and at a higher level develops into a very neat and symmetrical *amphivasal* bundle, i.e. possessing central phloem and external xylem. It is seen to be opposite an invagination of the central cylinder of the stem. At successively higher levels that portion of the xylem of the medullary strand which is opposed to the invagination becomes less and less in amount as the medullary strand approaches it. In its centre ground-tissue (cortical parenchyma and collenchyma) appears. The medullary strand then gradually unites with the central cylinder. Just before the phloem tissues of each mutually fuse, an endodermis arises within that of the medullary strand. After complete union between the vascular tissues and cortex of the central cylinder and medullary strand, a central strip of epidermis and, finally, a central space develops in the centre of what was once the cortex of the medullary strand. Slightly higher, the epidermis becomes continuous with that of the stem, and the central space with the outer air.

The union of the medullary strand with the central cylinder takes place at a point where the first forking of the stem is, at least partially, initiated.

The structure just described would result in the formation, as viewed from the outside, of a lateral pocket extending into the pith and narrowing to a point downwards and inwards (Text-fig. 1).



Text-fig. 1. Longitudinal section of portion of the stem showing one of the lateral pockets which give rise to "ring-fasciation." (Diagrammatic.)
x = xylem; *ph* = phloem; *c* = cortex; *m* = pith.

Plant 123 A

About $1\frac{1}{2}$ in. above the base of the stem, a minute, rudimentary amphivasal bundle arises *de novo*, and soon divides into three or four which, at a higher level become reduced to two only, both uniting with the central cylinder on one side at the point where an invagination has proceeded some way towards inaugurating a forking of the stem; neither medullary strand appears to develop any central cortical tissue or air space.

Above the region where this first forking occurs another medullary bundle arises *de novo* in the pith centre of one of the two branches; while, at a somewhat higher level, a bundle appears in the pith centre of the other branch and divides into two. The first-mentioned

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bundle gradually increases in size upwards, becoming very large before uniting with the invagination which represents the point of separation of a smaller branch.

Plant 123 B

The lower three inches of the stem show no medullary bundles. Then a minute strand arises in the pith centre. A few internodes above a second, very rudimentary strand, appears which, at a slightly higher level, dies out close to the vascular ring. The first-mentioned bundle gradually grows larger while approaching the ring. It has at first central xylem, but soon becomes amphivasal, containing central phloem and at about $1\frac{1}{2}$ in. above the point of its first origin, it unites with the ring at a node where a small axillary branch is given off. There was no trace of any medullary bundles in higher regions of the stem.

In 123 M and N the stem was fasciated, but very narrow in diameter at the base and much flattened and twisted above. It is not at all the type in which ring-fasciation would be likely to occur. The structure is quite normal.

Plant 125⁸

(The 125 brood was the result of crossing a "wild" normal plant with 29¹.)

At about the level of the first node of the stem, one large amphivasal and several minute medullary bundles arise *de novo* in the accurate centre of the pith. These then branch up into a larger number, and some more small ones arise *de novo*. Most of the bundles persist right up to where multiple forking takes place. About this region there occur two large bundles containing central cortex and epidermis.

Numerous small bundles are scattered in the pith. One of the large bundles unites with a prominent invagination of the ring; the other one doubtless did the same at a higher level, but was not traced upwards.

The largest of the small, scattered bundles above mentioned enlarges into an amphivasal one, developing central cortex and epidermis, and unites with the ring after two or three of the adjoining small strands have fused with it. Other tiny strands die out *in situ*.

The ring-fasciation structure of this stem is very well developed, arising as it does independently at two or three different regions of the pith, and culminating at different levels of the stem. The small,

rudimentary strands may evidently be regarded as so many *abortive ring-fasciations*. Each would represent, on the one theory of this phenomenon, the very intimate fusion of a small branch with the main stem, or, on the other theory, the baulked effort of the stem to separate off a small branch (see the discussion below).

125¹, 125², 125³, 125¹⁰ were all fasciated plants whose internal structure was quite normal, showing no trace of ring-fasciation.

Plant 56¹

In this stem the ring-fasciation shows much the same features as in that of previous stems described. The large amphivasal medullary strand acquires central cortex, epidermis, and air cavity some time before it unites with the invagination of the vascular ring. This invagination here, as in all cases, represents a longitudinal groove on the stem corresponding to the one-sided commencement of forking.

Plant 58

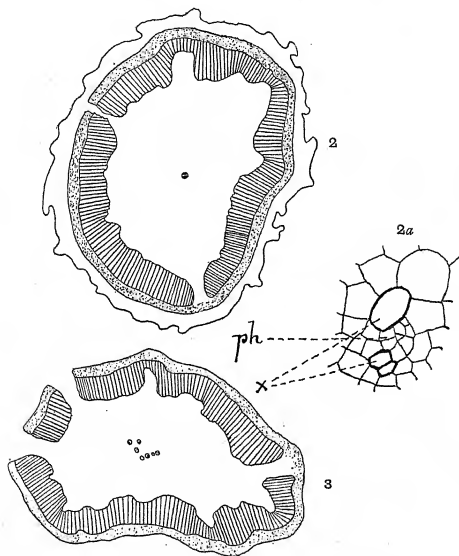
In this stem at first a single bundle, thereafter six or seven small bundles, arise *de novo* in the pith centre (Text-figs. 2, 2 a, 3). One of the bundles develops ahead into a large amphivasal structure, while the remaining ones unite with it at one side (Text-figs. 4, 5). The central large strand, before leaving the pith centre, develops a central air cavity, and epidermis and cortex in the usual way (Text-figs. 6, 6 a). One of the sclerotic strands adjoining it develops into a bundle and, shortly after the large concentric strand has united with the vascular ring in the orthodox way (Text-fig. 7), this second bundle (Text-fig. 7) unites at a slightly higher level with a bulge in the ring.

The diagrams of the ring-fasciation in this plant are typical representations of what occurs in all cases examined in other plants.

DISCUSSION AND CONCLUSIONS

As the fasciated stem replaces the whole, or nearly the whole, basal branching system, which is the normal, original condition, the individual components of which are very narrow in diameter, the ring-fasciation might be regarded as the result of a *congenital* fusion of all or most of these branches, and the forking, at successive levels, of the stem as the resolution of the latter into the former. But this last-named process never becomes completed, either as regards the actual process of segregation of each individual branch, or as regards the number of branches thus separated off.

In those cases of fasciated stems in which the forking occurs, not by means of ring-fasciation, but by simple abstriction, caused by two horizontal invaginations of the external tissues meeting on opposite sides of the stem (which is the mechanism of *all* ordinary



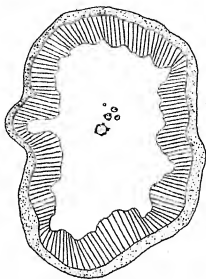
Text-fig. 2. Transverse section of stem near the base, showing the single central bundle. \times about 7. (Diagrammatic.)

Text-fig. 2 a. Medullary bundle of Text-fig. 2. \times about 60.

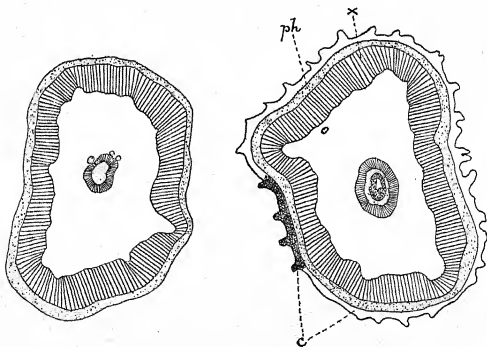
Text-fig. 3. Transverse section of stem near the base, showing a group of small central bundles. \times about 7. (Diagrammatic.)

forking) (Text-fig. 8), the congenital union of the lateral branches with the main axis would have to be regarded as very much more intimate, so that no internal trace thereof whatever (in the form of ring-fasciation) has been left.

However, on the whole, I do not think that the congenital fusion theory is the best explanation of the phenomenon. It seems best to



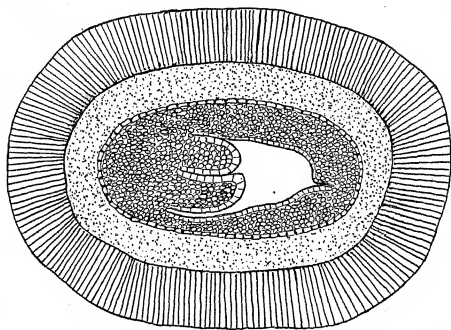
Text-fig. 4. Showing one of the bundles of pith increasing in size and becoming amphivasal. \times about 7. (Diagrammatic.)



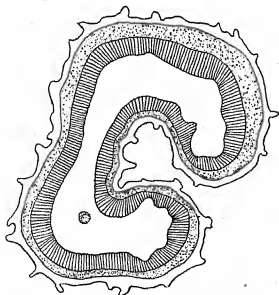
Text-figs. 5 and 6. Showing later stages in the development of the central large strand which forms the ring fasciation. \times about 7. (Diagrammatic.)

regard the fasciated stem as a *single* structure which is due to specially luxuriant growth. But it is to be held as being the *equivalent of the main stem, along with its numerous basal lateral branches, in the normal*

plant. That is, that the fasciated stem contains the lateral branches *in potentia* within itself, and endeavours in its upper part to give expression thereto. It seems impossible to imagine any fusion as having taken place.



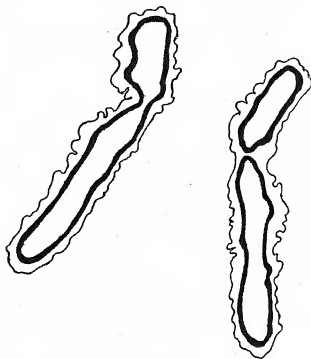
Text-fig. 6a. The central strand of Text-fig. 6 more highly magnified, showing the internal air-space, epidermis and cortex. (Diagrammatic.)



Text-fig. 7. The central strand is here shown uniting with the external stem-tissues, at the point which represents the aperture of the pocket. A second "ring-fasciation" is beginning. \times about 7. (Diagrammatic.)

Again, it might be surmised that the curious pocket- or funnel-formation which is generally the result of "ring-fasciation" and which is well-marked in the pea and dandelion, might be due to invagination from above downwards of the external tissues, just as often happens in a glove-finger. And, indeed, as I have indicated elsewhere, this is probably the *morphological* interpretation of the phenomenon, the invagination occurring at different points along the stem.

Ring-fasciation reproduces the structure which would have appeared had fusion between the stem and branches taken place



Text-fig. 8. Transverse sections of a branch at different levels, showing ordinary fasciation, and simple forking. $\times 10$. (Diagrammatic.)

in a particular way; while it is also the equivalent of, and reproduces ontogenetically, the structure which appears when vertical invagination of the tissues occurs congenitally.

The cases in the dandelion observed by Reichardt and Michaelis strongly support the view that the phenomenon is due to branching; the ring-fasciation there cannot be due to congenital fusion of branches, for branching is normally unknown in the dandelion scape. In these cases it is best regarded as a curious mode by which abnormal branching of the scape is effected.

The final conclusion, therefore, is that from the developmental point of view all stems exhibiting ring-fasciation are primitively

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simple structures which are in a gravid condition and which endeavour, at the earliest possible period, to deliver themselves of their progeny, the branches. They effect this by means of the peculiar process which we term "ring-fasciation." But why some of these gravid stems should branch up by means of ring-fasciation while others, having exactly the same external appearance, should do so by ordinary fasciation, it is impossible to say.

There are no homologies for the ring-fasciation structure. It may occur in plants belonging to the most diverse natural orders. The pronounced medullary vascular structure of *Campanula carpatica* which, as seen from the detailed description given above, may sometimes take the form of small scattered bundles, would appear to bear no relation whatever to the normal medullary bundle structure characteristic of certain species of *Campanula*. *C. carpatica* is one of those species in which medullary bundles have never yet been found, and probably never will be. Those which occur in the fasciated stems of this species are not at all, as far as can at present be seen, homologous with the medullary strands of, e.g. *C. latifolia* or *C. Trachelium*, but have a quite different origin and meaning.

I am much indebted to Sir Daniel Hall and to Miss C. Pellew, of the John Innes Horticultural Institution, for the supply of material for this investigation, and to Miss Pellew for the excellent photographs of normal and fasciated plants.

This work was carried out in the Jodrell Laboratory, Royal Gardens, Kew, and I have to thank the Director, and also Mr Boodle, the Keeper of the Laboratory, for the facilities afforded me.

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THE PYCNIDIA OF THE RUST FUNGI

By W. B. GROVE, M.A.

THE discovery of the function of the uredineal pycnidia by J. H. Craigie, of the Dominion Rust Research Laboratory, Winnipeg, as announced in a letter to *Nature* on November 26, 1927, will prove one of the most far-reaching of all the discoveries made on that group of Fungi. Before treating of one of the aspects of this discovery, it will be helpful to consider how it was made. The essence of the matter lay in the clever invention of the wonderfully simple method whereby monosporidial cultures could so easily be procured. This reminds one of the way in which W. F. Hanna, using his dry-needle method (*Annals of Botany*, 38, 791), obtained separate cultures from all four spores which had grown on a single basidium of *Coprinus lagopus* (*Annals*, 39, 448), with the aid of a process devised by Professor A. H. R. Buller in his laboratory at Winnipeg (see *Nature*, 114, 826). But there is one difference between the two methods which may be of great importance. In the method followed by Hanna all the four spores from each basidium could be separately treated; in the method used by Craigie for *Puccinia Helianthi* the sporidia were caught at random, and no one could tell exactly from where each one came. Two adjacent monosporidial cultures might have arisen from sporidia that had grown on different basidia. Presumably, if the origin of the sporidia could be made more definite, fresh light might be thrown upon the problem.

This thought has suggested to me a method (which I am now quite unable to carry out myself) whereby the four sporidia from a single basidium of a Uredine might with care be isolated without the slightest chance of admixture, and with as great a certainty as was done with the *Coprinus*.

If, towards the end of May, a leaf of *Euphorbia amygdaloides*, covered with the mature aecidia of *Endophyllum Euphorbiae-silvaticae*, is held for a few moments, with its lower surface downwards, over a gelatine film contained in a Petri dish, and then lightly tapped, a few of the ripe aecidio-teleutospores can be caused to fall upon the gelatine; a trial or two will easily decide the time of exposure, and the number of taps required. If the dish is then covered over and kept at a suitable temperature, the spores will germinate readily, sending out each a single basidium, which will produce its

four sporidia and shoot them off to a distance of a fraction of a millimetre, so as to fall upon the surface around the spore. If, therefore, the spores are scattered with sufficient sparseness, the four sporidia which came from each basidium can be easily identified. I have seen them thus shot off, each with its drop of water in the typical way, and afterwards lying as I have described, and sometimes even (owing to the curvature of the basidium) it might have been possible to decide with tolerable; though perhaps not with absolute, certainty which sporidium came from which basidial cell.

At this point it may be mentioned, parenthetically, that the spermatogones of the *Endophyllum*, which appear in April, mainly, but not exclusively on the upper surface of the leaf, have a sweet taste and a strong sweet smell, sweeter than that of *Puccinia obtegens*, and resembling that of the flowers of *Philadelphus coronarius*. Many small flies, beetles, and other tiny insects can be seen crawling over the spermatogones and sipping the juices.

The further procedure, viz. the culture of the sporidia on a living leaf of a healthy *Euphorbia*, would present little difficulty to an experienced uredinologist using the methods now in vogue. Or we might vary the method so as to dispense with the Petri dish, by letting the aecidio-teleutospores fall upon a moist leaf of the *Euphorbia*, germinate there and discharge their sporidia *in situ*, and thereafter produce pycnidia exactly as in Craigie's experiments. This method might be made to lead to some very pretty variations.

The object of these experiments would be to decide if the four sporidia on a single basidium are each of a definite (+)-ness or (-)-ness, as are the spores of *Coprinus Rostrupianus* (Miss D. Newton, in *Annals of Botany*, 40, 105), or possess a greater complexity (four "sexes") as in *Coprinus lagopus*. There can now be little doubt that the pycnosporos, and therefore the sporidia, of *Puccinia Helianthi* and *P. graminis* have sex.

These experiments may well lead us, on reflection, to envisage in our minds the following probable conclusions. Each sporidium, on germination, produces a mycelium which has a definite (+)-ness or (-)-ness, and this mycelium produces conidia (pycnosporos) that have a similar distinction of character. If by any means, as by insects, these conidia of opposite sexes are mixed together, they produce, in some way not yet worked out, a spore-bed on which hyphae belonging to each of these groups grow up together side by side, and finally some of the upper cells of these hyphae fuse with one another in the way discovered by Blackman (1904) or by Christman (1905).

Between these two ways there is no essential difference; all that is needed is the fusion of a (+) cell with a (−) cell, so that the two nuclei may range themselves side by side with each other, and thenceforth behave as conjugate nuclei in the well-known fashion. The fusion is not, as Blackman taught, the fusion of two *female* cells as a *substitute* for fertilisation, but a real fertilisation process involving opposite sexes. It seems to me that the investigation of the behaviour of the four sporidia from a single basidium of the *Endophyllum* on *Euphorbia* would in time enable this problem to be definitely solved.

BOTANICAL DEPARTMENT,
UNIVERSITY OF BIRMINGHAM.

FIFTH INTERNATIONAL BOTANICAL CONGRESS

CAMBRIDGE (ENGLAND) 1930

We have been asked to publish the following notice:—

Motions on the subject of Nomenclature for consideration by the Congress should be in the hand of the Rapporteur général, Dr John Briquet, before *September 30, 1929*.

Motions must be presented in the form of additional articles (or amendments) to the Rules of 1905–1910, drawn up in the form adopted in the *International Code*, and must be drafted as briefly as possible in Latin, English, French, German, or Italian. At least 100 printed copies must be presented.

According to the decisions of the Brussels Congress 1910, only motions relating to new points which were not settled in 1905 and 1910 can be presented. Motions which do not answer to these conditions can only be discussed if the Cambridge Congress decides to take them into consideration.

For further information about the programme of work in nomenclature, apply to the Rapporteur général, Dr John Briquet, Conservatoire botanique, Geneva (Switzerland).

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THE ENCRUSTING ALGAL COMMUNITIES OF CERTAIN FAST-FLOWING STREAMS¹

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(With Plate V and 10 figures in the text)

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(I) INTRODUCTION

THE investigations upon which this communication is based were conducted in September of 1918 and 1920 on a number of rapidly flowing streams between Lynton and Ilfracombe on the north coast of Devonshire. It is natural that, apart from a plankton which has not been examined and is likely to be very meagre, the entire vegetation of such rapid streams consists of attached forms. These comprise two distinct biological types, the encrusting completely adhesive types and those in which the greater part of the body projects or even trails out into the current. To the latter, which are often well

¹ From the Botanical Department, East London College, University of London.

developed in the full rush of the torrent, belong a number of filamentous algae (short tufts of *Cladophora glomerata* Kütz.¹, a short-celled sterile *Oedogonium*, *Ulothrix zonata* Kütz., *Lemanea* spp., etc.) and the mosses, to the former the aquatic lichens and some very characteristic algal types. In the present paper it is proposed to deal only with the encrusting algal communities. The disentanglement of the forms concerned has proved to be a matter of considerable difficulty and is responsible for the great delay in publication. The lichens have been dealt with by Watson⁽¹⁷⁾; in the course of this work only species of *Verrucaria* (mainly *V. submersa* Schaer.²) have been certainly identified.

At the outset it must be emphasised that only those forms are considered that grow submerged on the pebbles and boulders or on the *Cladophora* in the rapidly flowing water. Those that occur at the sides of waterfalls, which are at times reached only by spray and are partly subaerial, and those that are to be found in the occasional quiet water near the banks (e.g. *Nostoc* spp.) are not taken into account. There remains a set of forms that are subjected to very definite and extreme conditions, viz. constantly moving and well aerated water and the very considerable scour of the current. Except after heavy rains when much sediment is carried, the water is usually clear and transparent. All the British streams that have been specially investigated arise on Exmoor and flow over sandstones and shales; the water is therefore probably somewhat acid and non-calcareous.

Three types of encrusting algal communities can be distinguished on the submerged rocks and pebbles, viz. (1) the *Hildenbrandia-Lithoderma* community, (2) the *Chamaesiphon* community, a few representatives of which also occur on *Cladophora*, and (3) the *Phormidium* community. Apart from the *Hildenbrandia*, the *Lithoderma*, and a rather rare species of *Gongrosira*, all the encrusting algal forms belong to the Myxophyceae. The only Diatom that is of any considerable importance is *Cocconeis placentula* Ehrenb., the flat frustules of which are to be found everywhere on the rocks, usually overgrown by the other forms, but sometimes adhering to them.

¹ Not observed in the slow-flowing Badgeworthy Water.

² I am indebted to Dr W. Watson for this determination.

(2) THE *HILDENBRANDIA-LITHODERMA* COMMUNITY

This is composed essentially of the red alga *Hildenbrandia rivularis* (Liebm.) Bréb., but it has nearly always been found associated with the yellowish-brown crusts of a form of *Lithoderma fluviatile* Aresch. (cf. also Skuja (16), pp. 663, 665), the latter often overgrowing the former; species of *Verrucaria* are likewise often present (cf. Budde (2), p. 286). At first sight *Hildenbrandia* appears to be a rare alga in the streams under consideration; thus, for example, it is not at all conspicuous in the East Lyn and was overlooked altogether in 1918. The best developed specimens are, however, to be found underneath overhanging ledges of rocks or on the under side of projecting pebbles, wherever there is some slight current. It is obvious from this characteristic distribution that *Hildenbrandia* tends to develop best in the shade, and this is supported by the fact that in the River Heddon (above Hunter's Inn) and especially in the River UMBER, both of which are more shaded¹ than the East Lyn, it was relatively more conspicuous and not so often hidden away on the underside of the rocks. *Hildenbrandia* was not observed in the part of the River Heddon lying between Hunter's Inn and the sea, where there is practically no shade. This quite agrees with what is known as to the mode of occurrence of this alga on the continent (cf. Lingelshheim and Schröder (7), p. 271; Skuja (16), p. 660). As a general rule *Hildenbrandia* and the associated *Lithoderma* are not found growing at the points of most rapid flow, but in the River Heddon the former was frequently noticed attached to the side of the rock facing towards the current (i.e. the same situation as that frequented by *Phormidium*, cf. p. 191) and often almost in the full force of the latter. According to Allorge (11), p. 120) *Hildenbrandia* is confined to siliceous torrents (cf. however Skuja (16), p. 664).

(a) *Hildenbrandia rivularis* (Liebm.) Bréb.

(Pl. V, phot. 3; Fig. 1)

The thallus of this alga is completely encrusting. Smaller thalli are often nearly circular (Pl. V, phot. 3) and such may attain a diameter of 2.5 cm., but the larger ones are of very irregular shape and these often cover several square centimetres of rock-surface. They have never been found on any other substratum. It is probable that some of the larger thalli are formed by the confluence of smaller

¹ Parts of the UMBER are subterranean, whilst others are so overgrown by shrubs as to be practically hidden.

ones (Skuja 1961, p. 662). The margin of the thallus is generally fairly regular, but the surface is mostly uneven and sometimes appears to be composed of numerous irregularly overlapping scales. The colour is a bright crimson and no brownish shades were observed.

The thallus consists of vertical rows of flattened or squarish cells (diam. $7-8.5\mu$, Fig. 1 D) which fit very compactly together and arise from a basal stratum. The ends of these rows, forming the upper surface (Fig. 1 A), present the appearance of a dense pseudo-parenchyma composed of polygonal isodiametric cells, usually with rather

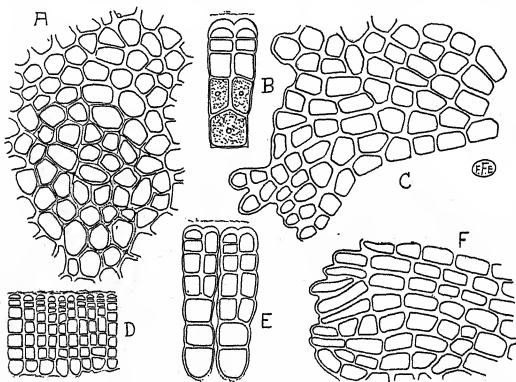


Fig. 1. *Hildenbrandia rivularis* (Liebm.) Bréb. A, upper; C and F, lower surfaces of the stratum; D, small part of a stratum in section; B and E, the same enlarged. (D $\times 350$, other figures $\times 700$.)

strongly thickened walls. In the underlying layers the intervening walls are not so thick. The basal layer of the thallus adjacent to the substratum (Fig. 1 C, F), when viewed from the surface, consists in great part of rather elongate cells radiating more or less definitely from some central point, so that its origin from a series of completely coalesced filaments is clear. The cells are in most cases specially elongated towards the margin and here such long cells may also be observed on the upper surface. From each cell of this parenchymatous basal stratum an upright row arises. That these rows, though in very close contact, are actually independent of one another is seen

by the ease with which they will separate when the thallus is subjected to slight pressure. Some of the upright rows are simple, but many of them dichotomise (Fig. 1 *B, D, E*), and so the elongate cells of the basal stratum are replaced by isodiametric ones at the top. The growth of the thallus is probably essentially marginal, and in most cases the edge consists only of two superposed layers. The upright rows probably increase in height by intercalary division.

The top cell of each row frequently has a rounded outer face, though it is sometimes almost flat, and the external wall is usually more strongly thickened than the others (Fig. 1 *B, E*). Beyond the outer wall is a moderately thick layer of mucilage, which may in rare cases be absent. Quite frequently these superficial cells are colourless, and similar hyaline cells often terminate the free margin of the thallus. The cells of the basal layer, as seen in section, vary with the surface of the substratum. When the latter is smooth, they appear flat with plane lower walls, but when the substratum is at all uneven they are produced into short colourless rhizoid-like outgrowths which fit into the irregularities of the substratum. A denser round body occupying the centre of the cell is no doubt the nucleus (Fig. 1 *B*), but no details as to the chromatophores could be recognised.

When the thallus presents the scaly appearance mentioned above, the scales seem in part at least to be portions of the thallus which are becoming detached. They usually consist of cells with deep red contents and have an irregularly lobed margin as though abundant growth were taking place there. This is therefore probably a method of vegetative reproduction, but no other reproductive structures were ever observed, nor are any yet certainly established for this species. Budde's (2,3) account of the reproduction of this alga is little convincing (see also Skuja (16), p. 667).

Associated with the *Hildenbrandia* are the thalli of *Lithoderma* and some individuals of *Cocconeis placentula*, whilst in some parts of the Umber the thalli were densely covered with *Oncobyrsa cesatiana* Rabenh. In many cases, however, they were quite clean.

(b) *Lithoderma fluviatile* Aresch.

(Pl. V, phot. 5; Fig. 2)

This species was found especially abundantly in the Umber, where it occasionally occurred by itself, but mostly, both here and in the East and West Lyn, it grew on the same stones as bore *Hildenbrandia*. The thalli were always small and rather irregular (Pl. V, phot. 5) and

yellowish brown in colour. In this last respect the British form differs from the published descriptions of *Lithoderma fluviatile* which give the colour as olive green to black (cf. Pascher (11), p. 128). The construction is very similar to that of *Hildenbrandia*, but the cells are far larger.

In this case the basal layer in contact with the substratum shows a much more definite arrangement of the cells in rows (Fig. 2 K), so that the filamentous nature is more apparent. The structure is, however, quite compact, even at the margin of the basal layer which is irregularly lobed. The dense vertical rows consist of flattened cells

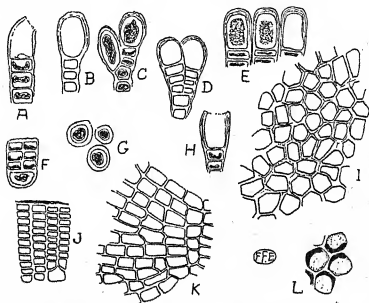


Fig. 2. *Lithoderma fluviatile* Aresch. A-E and H, upright threads with sporangia; in A and H showing dehiscence, in B a dense soral arrangement. F and J, parts of the strata in section showing the arrangement of the vertical cell rows. G, sporangia from the surface. I, upper, and K, lower surfaces of a stratum. L, some of the cells of I, showing contents. (J \times 250, all other figures \times 375.)

and show occasional forking (Fig. 2 F, J). The upper layer of the thallus is a compact pseudo-parenchyma of isodiametric polygonal cells (Fig. 2 I), which all lie at the same level giving a smooth surface. The cell walls are thick throughout; those of the basal and superficial layers are usually yellow or yellowish brown in colour, but those in the intervening layers are colourless. The cells appear to contain only a single brown chromatophore (Fig. 2 L).

Many of the thalli seem to be only two or three layers of cells thick (cf. Fig. 2 F), but sometimes the vertical rows comprise a much larger number of cells (Fig. 2 J). In such cases the inner cells are often empty or contain disintegrating contents. On the other hand,

the superficial cells often have dense contents. The dimensions are: basal cells 10–17 μ broad and 17–20 μ long; superficial cells 10–19 μ broad.

Many thalli were found producing sporangia, which appear irregularly circular when seen from the surface (Fig. 2 G) and oblong, clavate, or globose when viewed from the side (Fig. 2 B–D). The production of sporangia appears to commence in the centre of the thallus, but may ultimately involve a large part of the surface. When this is the case the superficial layer presents a much more irregular appearance than that of the vegetative thallus, and the projecting sporangia are seen not to fit so closely together; occasionally, however, they were found in compact rows (Fig. 2 E). As a general rule the sporangia arise at the ends of the vertical threads (Fig. 2 A, B, D, H), although sometimes a sporangium is found as a lateral outgrowth of one of the lower cells of the upright rows (Fig. 2 C). The sporangia are always appreciably larger than the ordinary cells (20–27 μ broad and 26–34 μ long). They have thick white obscurely stratified walls and dense brown contents; the latter occasionally appeared divided into a number of portions, but the preservation of the material was not adequate for any detailed study of this matter. In many cases the sporangia were empty, with the upper part of the wall irregularly broken away (Fig. 2 A, H).

Apart from the difference in colour of the thalli, which was noted above, the British alga differs in the larger dimensions of the cells, the usually more elongate shape of those composing the basal stratum, in the thick walls of the sporangia, and the yellow or brown colour of many of the walls of the vegetative cells, from the hitherto published descriptions of *Lithoderma fluviatile*. These differences may mark a distinct species, but the other freshwater members of the genus are at present so imperfectly known (cf. Pascher(11), p. 128) that it was considered undesirable for the present to establish a separate species. This is the first record of the occurrence of a freshwater member of Phaeophyceae in the British Isles.

(3) THE CHAMAESIPHON COMMUNITY

(Pl. V, photos. 1, 2, 4)

Many of the pebbles and smaller rocks, both in the swiftly flowing water and more strikingly so in the somewhat slower flowing stretches, bear patches of encrusting algae, nearly all of which are blue-green forms and the majority of which belong to the Chamaesiphonales (cf. Rabanus(12), p. 15). Where the current is very strong, the growth

is not so considerable and usually of a more or less greenish hue, but elsewhere the pebbles bear conspicuous sepia brown or brownish black patches (Pl. V, photos. 1, 4), which sometimes have a definite reddish tint, and a more or less slimy character; they dry almost black. In many cases these patches form a very prominent speckling on the rock surface, covering the greater part of it, and this type of growth was observed in all the Devonshire streams examined, with the exception of the River Uمبر. The small patches are roughly circular and up to 5 mm. in diameter, but most are larger and irregular in shape and are no doubt formed by the confluence of smaller ones. Occasionally the patches are elongate, their long axis being parallel with the current (Pl. V, phot. 2). The crust of alga is not of any appreciable thickness, probably never exceeding 100μ and no doubt usually much thinner. Many of these crusts are composite, consisting of several algal forms, but even when only one form is present it is scarcely possible to distinguish it macroscopically from another.

The commonest forms involved in the production of these crusts are *Chamaesiphonopsis regularis* nov. gen. et sp. (Fig. 3 A-I), *Chamaesiphon ferrugineus* n. sp. (Fig. 5), and *C. pseudo-polymorphus* n. sp. (Fig. 4). All of these appear at times to form practically pure crusts, although often associated together. In Badgeworthy Water *Chamaesiphon ferrugineus* is the most abundant, in the River Heddon the red form of *C. pseudo-polymorphus*, whilst in the East and West Lyn the latter is common, but somewhat outnumbered by *Chamaesiphonopsis regularis*. A very frequent form in many of the rivers is *Chroococcopsis fluminensis* n. sp. (Fig. 7), which occurs as part of the green covering in the very rapid current, but elsewhere is usually overgrown in most places by the three first-mentioned algae. Side by side with the *Chroococcopsis*, and in the rapids even more common than it, is found *Pseudoncobyrsa fluminensis* n. sp. (Fig. 6). This form also grows amid the crusts of *Chamaesiphonopsis*, where it is associated with *Phormidium foveolarum* (Mont.) Gom. (Fig. 3 M), the only member of the Hormogoneales that plays any rôle in the *Chamaesiphon* community. Its threads, however, are very frequent, both among the growth in the rapids where they form vertical tufts attached to the rocks, and in the compact crusts found in slower-flowing parts.

A much rarer form is *Oncobyrsa rivularis* Kütz. (Fig. 8 A-E), which has been mainly observed as an epiphyte on *Cladophora*, where it is associated with *O. cesatiana* Rabenh. (Fig. 8 F-H) and *Xeno-*

coccus chroococcoides n. sp. (Fig. 9 A-K). The last two have only been met with very rarely on stones, but this may be an oversight. Other members of the encrusting community on the *Cladophora* are *Chamaesiphon curvatus* Nordst. (Fig. 9 L-P) and radiating tufts of *Ankistrodesmus falcatus* (Corda) Ralfs var. *stipitatus* (Chod.) Lemm. (the latter also very rarely on rocks in the full torrent), as well as threads of *Chantransia* spp. It is noticeable that in the East Lyn, and no doubt also in the other streams, the diverse blue-green forms and the *Ankistrodesmus* were common on the *Cladophora* only at the points of less rapid flow. Here they are associated with abundant *Cocconeis placentula*. In the more rapidly flowing parts, however, Diatoms (especially *Gomphonema olivaceum* Kütz.) play a more important part.

On most of the rocks and pebbles the diverse blue-green forms producing crusts are accompanied by the Diatom *Cocconeis placentula* Ehrenb., whilst in the green growth found in the very rapid water a new species of *Gongrosira* (*G. fluminensis* n. sp., Fig. 10) is not uncommon.

In the following pages the different members of the *Chamaesiphon* community are considered in detail.

(a) *Chamaesiphonopsis regularis* n. gen. et sp.

(Fig. 3 A-I)

It is only after very considerable study that it has been possible to separate this form from the many others with which it occurs associated, and the writer regrets that he has been responsible for the creation of two synonyms in earlier references to it. It has been recorded and figured in *British Freshwater Algae* (2nd edit. p. 467) under the name *Xenococcus britannica* and had previously been referred to as *Oncobyrsa britannica* (*Journ. of Ecol.* 13, 1925, p. 173). The specific name here adopted is a more suitable description than the purely geographical one.

This alga possesses a parenchymatous basal layer composed of closely fitting polygonal cells with firm but thin membranes which form a prominent network (Fig. 3 A, I). The mode of development of this basal stratum is obscure, but the cells are seemingly produced by division from one another; as a general rule the cells are remarkably alike in form and dimensions (diam. 4.5-5 μ) and show no definite arrangement, although in a very few cases there was some indication of an orientation in rows giving a faint filamentous appearance. From this basal layer there arise dense upright rows of elongate, oblong cells, usually at least twice as long as broad, those in

the centre of the patch being vertical, those at the edge radiating outwards (Fig. 3 *F*). In general there would appear to be several cells in the rows (Fig. 3 *H*), but in young growth there are only two (Fig. 3 *E*) or even only the basal layer. The upper cells are devoid of an obvious membrane, and probably in most cases this is true of all the cells of the rows except for the basal ones. The regularity of

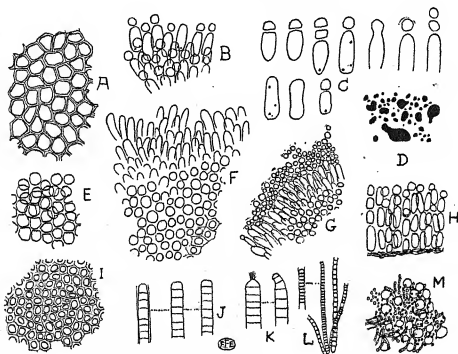


Fig. 3. *A-I*, *Chamaesiphonopsis regularis* n. gen. et sp. *A* and *I*, portions of the parenchymatous basal stratum; *B*, edge of a stratum showing the elongate cells and the abstriction of gonidia; *C*, various stages in the production of the latter; *D*, a few of the patches formed by this and other forms; *E*, surface view of a young crust, showing the polygonal cells of the basal stratum and the rounded cells of the second layer; *F*, part of the edge of a crust, showing cells in side view towards the edge and elsewhere in surface view; at the lower end some of the cells of the underlying stratum are seen; *G*, edge of a crust, showing abundant production of gonidia; *H*, section of a crust. *J*, *Phormidium retzii* Gom., tips of three trichomes, from the East Lyn. *K*, *P. autumnale* Gom., tips of two trichomes, from Badgeworthy Water. *L*, *P. foveolarum* (Mont.) Gom. *M*, small part of a stratum of *Chamaesiphonopsis regularis* with numerous threads of *Phormidium foveolarum*. *A*, *C* $\times 750$; *B*, *E*, *H* $\times 640$; *D* slightly magnified; *F*, *I-M* $\times 550$; *G* $\times 340$.

the basal layer extends also to the vertical rows to which it gives rise, and the exceedingly uniform groups of projecting elongate cells of this form are in marked contrast to the irregularity of the stratum of *Chamaesiphon pseudo-polymorphus* with which it is often associated. When a stratum is viewed from the upper surface the cells appear circular and, although rather densely crowded, they are not

in lateral connection with one another (Fig. 3 F), a feature which contrasts with the aspect of the basal layer where the cells are united.

The upright rows of cells no doubt originate for the greater part by the germination *in situ* of gonidia, budded off from the apices of the cells (Fig. 3 C). It would seem that the cells of the basal layer, elongating at right angles to the substratum, produce apical gonidia by abstriction as in a *Chamaesiphon* and that simultaneously the apical portion of the membrane becomes mucilaginous. The gonidia, thus formed, elongate, without becoming enveloped by a fresh membrane, and these cells in their turn produce gonidia and thus the several-layered stratum originates. There is no doubt a good deal of structureless mucilage between the cells. The gonidia are usually of practically the same width as the cell from which they are abstricted and spherical in form (Fig. 3 B, C). Several may be produced in succession from the same cell and, when this occurs extensively, large numbers of gonidia may be found accumulated at the surface and along the free edge of the strata (Fig. 3 G). Such strata often show a faint irregular layer of mucilage beyond the mass of gonidia.

All the cells composing the strata of this form are of a pale blue colour, with non-granular or only slightly granular protoplasts, although one or two larger granules are often to be found, especially at the base of the cell (Fig. 3 B, C). The usual dimensions are: diam. basal cells, 4.5-5 μ ; diam. projecting cells, 3.5-4.5 μ ; length of projecting cells, 10.5-14 μ .

Chamaesiphonopsis is thus a *Chamaesiphon* in which the gonidia germinate *in situ* and in which large numbers of gonidia-producing cells are united to form a compact basal stratum. The outstanding feature is the tendency to develop upright rows, and this form is thus comparable to *Chamaesiphon oncobyrsoides* Geitler (4), p. 330), which should probably be transferred to this genus. It may be pointed out that the formation of a spherical gonidium by apical abstriction is but a special case of ordinary cell division in the Myxophyceae, but whereas in the latter case the cell becomes constricted into two equal parts, in gonidium formation the parts are unequal. When the gonidium grows *in situ* into a new cell, we are really only dealing with a case of unequal cell division. It is not improbable that equal cell division may occasionally occur in *Chamaesiphonopsis regularis* (cf. the lower middle cell in Fig. 3 C), but this is certainly rare. If it were frequent, one would not hesitate to consider this type as a filamentous one, and there is no reason against such an

interpretation even when the bulk of the division is unequal. *Chamaesiphonopsis* may thus be regarded as a connecting link between the Chamaesiphonaceae and the Pleurocapsaceae.

(b) *Chamaesiphon pseudo-polymorphus* n. sp.

(Fig. 4)

This form produces much more irregular and often less compact strata than the last, the irregularity extending both to the shape of the cells and to their grouping. Apart from this it is readily picked out by its rather larger cells (diam. $6-8\mu$) and the invariable presence of a well-defined membrane or sheath around them. Seen from the surface the cells appear round, with a very pale blue homogeneous protoplast surrounded by a firm smooth membrane (pseudo-vagina¹) which is sometimes slightly yellow or brownish (Fig. 4 H). The cells are by no means of equal size, although large ones preponderate. The majority of the cells, when seen from the side, are rather short ($9-10\mu$ long) or even squat, and this applies particularly to those which are producing gonidia (Fig. 4 B, F, M). The membrane in such cases forms a wide open, more or less cup-shaped, pseudo-vagina which in rare cases (Fig. 4 F, a) may be thickened. At the same time occasional elongate cells are to be found (Fig. 4 A, I-K) and such may in some strata be in the majority (Fig. 4 H, N). The gonidia are mostly smaller than the cells from which they are abstricted and, though not rarely spherical, are more usually hemispherical or flattened (Fig. 4 F, J, K). Especially the short type of cell producing gonidia varies very considerably in shape, a fact which will be sufficiently obvious from the figures (Fig. 4 F, M) without further description. Such cells commonly exhibit a prominent basal granule which is either median or displaced a little towards one side. Gonidia are often to be found in short rows (Fig. 4 F e, K, J, M c). An irregular arrangement, implying possible longitudinal division, is rare (Fig. 4 I, M a).

The large strata produced by this species frequently show an irregular basal layer formed of somewhat polygonal cells closely fitted together, in which, however, the individual cells are separated by slight spaces at the corners from their neighbours and do not form the compact and regular basal layer that is distinctive of *Chamaesiphonopsis regularis*. The strata consist usually of several layers of cells which, however, show no regularity in their arrangement. The different cells seem to dovetail in between one another in most cases

¹ Geitler (4), p. 322.

(Fig. 4 E, N), and it looks as though the gonidia had germinated after some slight displacement to one side, the individuals of one layer fitting loosely and irregularly in a haphazard manner between those of the one below. Only in rare cases were the successive cells found

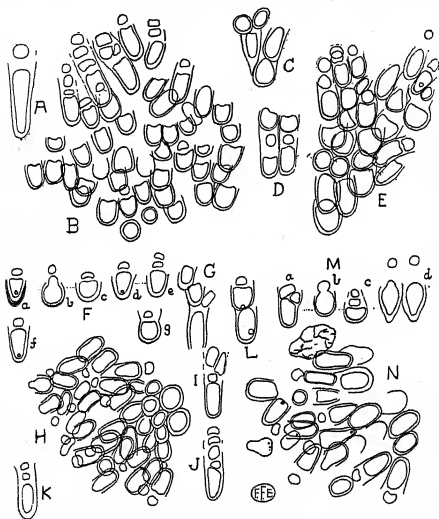


Fig. 4. *Chamaesiphon pseudo-polymorphus* n. sp. B, J and M are drawn from the red form found in the River Heddon, the others from the ordinary form. B, E, H and N, drawings of parts of various strata. A, elongate type of cell. C, G, L, colony formation. D, germination of gonidia *in situ*. F and M, diverse shapes of cell and types of gonidium formation. I-K, gonidium formation by elongate cells. (All figures $\times 740$.)

in rows (Fig. 4 D) and only in a few instances were indications observed of a *Dinobryon*-like grouping of the new cells at the mouth of the sheath of the old ones (Fig. 4 C, G). Such definite colony formation as Geitler (4) has described in various species of *Chamaesiphon* was quite the exception.

The lower cells of the strata are usually of the short squat type

and appear to have given up the production of gonidia, while the upper cells are often rather longer and actively engaged in gonidium production (Fig. 4 B).

In the River Heddou many of the strata were composed of cells with deep red contents, but no other difference between them and the ordinary pale blue form could be found. The few strata of *Chamaesiphonopsis regularis* occurring in the same situations likewise possessed red cells. It is not possible to relate this phenomenon to any special features of the habitat.

The new species of *Chamaesiphon*¹ just described stands nearest to *C. polymorphus* Geitler (4), p. 327), but differs in the possession of firm membranes (sheaths) around all the cells, in the absence of the cap of mucilage found at the apices of the cells of the latter species, in the far less definite method of colony formation, and in the fact that the gonidia are often less broad than the cells from which they are abstricted.

Both *Chamaesiphon pseudo-polymorphus* and *Chamaesiphonopsis regularis* are very abundantly colonised by *Phormidium foveolarum* (Mont.) Gom., whose rather fine (diam. 1.5μ) threads (Fig. 3 L) are to be found collected in tufts nestling between any gaps in the strata of these forms, and in some cases protruding everywhere between the cells (Fig. 3 M). In no case, however, was this species found forming independent strata of its own.

(c) *Chamaesiphon ferrugineus* n. sp.

(Fig. 5)

This form produces sepia brown patches on the stones in the rather slow-flowing Badgeworthy Water (Pl. V, phot. 4), where it is seemingly the commonest type, but it also occurs in the East and West Lyn. It forms small, very irregular patches.

The cells are usually oblong, from one and a half to two times as long as broad, and there is not very much variation in shape. The sheaths are goblet-shaped and, in the young condition, thin and barely coloured (Fig. 5 G n), but always quite distinct. Later they become markedly thickened and acquire a deep brown colour due to deposition of iron, chiefly in the ferrous and partly in the ferric state² (Fig. 5 D, F, etc.). Seen from the surface, both the proto-

¹ The same species, though with rather smaller cells, has been found on the stones in a rapid stream (Roth Murg) in the northern part of the Black Forest, here accompanied by a form closely resembling *Coccomyxa subglobosa* Pascher (10), p. 210.

² I am indebted to Dr F. M. Haines for this information.

plasts and the enveloping sheaths appear circular (Fig. 5 A), and there is generally among the members of a stratum considerable variation in the thickness and the depth of pigmentation of the sheaths. The individual cells are usually clearly marked off from one another and, though dense strata are formed, the basal layer is not as compact as in the last-described species. In old strata, however, the entire space between the cells may be occupied by the dark brown deposit (Fig. 5 B). In many, but not in all, individuals the sheaths are provided with a basal process resembling a small stalk (Fig. 5 C, D, P), the origin of which is not clear.

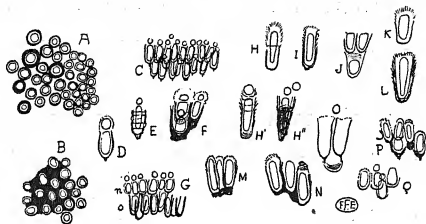


Fig. 5. *Chamaesiphon ferrugineus* n. sp. A and B, surface view of two strata, of which B is the older. C and G, strata in section; in G the individuals of the upper layer (n) have not yet thickened their sheaths. D, gonidium formation. E, H, I, K, L, single cells showing structure of the sheath and gonidium formation. F, J, M-Q, various types of aggregates. (All figures $\times 440$.)

The strata commonly consist of two more or less regular layers of cells (Fig. 5 C, G), both of which may be abstricting gonidia. The upper layer is often composed of cells which have not yet developed the thick brown sheaths characteristic of the mature individuals (Fig. 5 G), and there can be no doubt that this second layer has originated by the germination of gonidia produced from the underlying one. It is not improbable that some of the strata consist of more than two layers, but the older ones appear to tend to collapse ultimately and probably in most strata not more than two living layers exist. It seems that, as the individual gets older, the basal part of the sheath becomes more and more thickened and infiltrated with the iron deposit (Fig. 5 F) until nothing remains except a brown irregular crust upon which the later generations lie (Fig. 5 M, N). Fig. 5 B may well mark a stage in this process, in which the proto-

plasts are still present within their sheaths, but later they would seem to disorganise. Many of the sheaths of the active individuals show a very distinct fimbriate structure and seem to grow by the successive deposition of inclined layers, the one above the other, the individual layers projecting at the surface and giving rise to the frayed appearance presented by the older sheaths (Fig. 5 *E, H, I, K, L*). It is noticeable that this frayed appearance is confined generally to the upper part of the sheath, while the lower unfrayed portion becomes steadily thicker and it is this part that alone persists in the final stage. The fimbriate structure may appear in the sheath before (Fig. 5 *M, H, I*) or after the production of gonidia has commenced.

It does not seem that there is any definite regularity in the arrangement of the successive layers of the strata. In a few cases one can find definite evidence that the gonidia have germinated within the aperture of the sheath of the parent cell (Fig. 5 *F, J, O*), but in the vast majority of cases it looks as though one generation merely grew on top of the preceding one, attaching itself at any suitable point, inside or outside the parent sheath (Fig. 5 *P*), or merely fitting between the individuals of the previous generation. The stratum is thus an aggregate like that of the previous species, but the successive generations are more definitely grouped in layers than they ever are in *C. pseudo-polymorphus*. On the other hand, the layering is not as definite as in *C. fuscus* (Rost.) Hansg. (Geitler⁽⁴⁾, p. 323).

The gonidia of *C. ferrugineus* are usually spherical and of about the same size as the cell from which they are abstricted (Fig. 5 *C, D, H', H''*, etc.). Like the protoplasts of the cells producing them they are very pale coloured and altogether homogeneous. It would seem that commonly only one or two gonidia are produced, but some of the longer cells appear to produce more (Fig. 5 *H''*). The dimensions of this species are: diam. cell with sheath, 3.5-7 μ ; diam. protoplast, 3.5-4.5 μ ; long sheath, 10-13 μ .

This species resembles *C. fuscus* (Rost.) Hansg. in the colour and structure of the sheaths, but the latter has much longer cells, more definite colony formation, a more evident layering of the strata, and probably a more abundant production of gonidia.

(d) *Pseudoncobyrsa fluminensis* n. sp.

(Fig. 6)

This species has been found especially in the East Lyn among the thin slimy green or brown covering on smooth rock surfaces in the midst of the rushing torrent. It occurs also among the denser growth of *Chamaesiphonopsis regularis* and *Chamaesiphon pseudo-polymorphus* on the stones in the less rapidly flowing regions, but it is not at all conspicuous there. It forms flat, rather extensive microscopic crusts (usually up to 70μ thick) consisting of immense numbers of parallel rows of minute, mainly elongate cells arising at right angles to the substratum (Fig. 6 A-C, E, I). The cells composing these rows are usually oblong with rounded ends (Fig. 6 F), but some of the cells in the rows are always shorter, often almost square; the cells are usually 2μ wide and $3.5-5\mu$ long. There is no evident basal layer from which the upright rows might originate, and it is not clear how the extensive strata are produced. Branching of the rows, if it occurs at all, is exceedingly rare. The consecutive cells of the rows are never in direct touch with one another, being always separated by slight interspaces, but the majority of the rows are clearly defined and there can be no doubt that the cells composing them have originated from one another by division. Transversely divided cells can be found in nearly every row (Fig. 6 F). Not uncommonly a faint delicate sheath can be distinguished enclosing the cells of a row (Fig. 6 A, H) or even enclosing each individual cell (Fig. 6 F), but often there is no sign of this. All the cells are, however, embedded in structureless mucilage which holds the rows together and in older strata is usually coloured a more or less distinct yellow. The irregular edge of this mucilage can mostly be detected just beyond the ends of the rows by the adherence of foreign matter (Fig. 6 A, C, I).

Whilst in many strata the cells remain of the same minute width throughout, in others, and especially those with yellow-coloured mucilage, the end cells of the rows are appreciably enlarged (diam. $2.8-4\mu$) and provided with firm yellow or brown membranes (Fig. 6 A, E, G, H, I). Sometimes these cells follow quite suddenly on the narrower ones (Fig. 6 A, E), in other cases there is a somewhat progressive enlargement, so that the cells beneath the terminal ones are more or less pear-shaped (Fig. 6 H, I). The fate of these enlarged rather thick-walled cells is not known, but they have most of the characters of spores and no doubt behave as such. The cell contents are, however, altogether homogeneous. They may occur singly at the ends of the

rows (Fig. 6 *I*) or may lie in short series (Fig. 6 *A*, *G*). In the ordinary vegetative cells, whose protoplast is a pale blue, there is very often a single conspicuous granule which is frequently found in the middle of the cell, but may lie at one end (Fig. 6 *F*, *H*). These granules are often very conspicuous when the cells are viewed from the surface (Fig. 6 *B*), but in the spores they are lacking (Fig. 6 *D*).

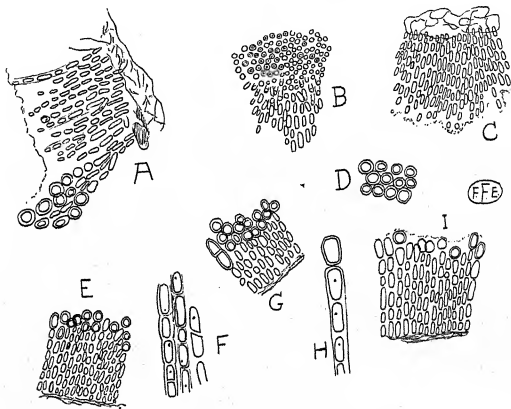


Fig. 6. *Pseudonocobyrsa fluminensis* n. sp. *A*, *C*, *E*, *G* and *I*, parts of various strata seen in optical section, in all except *C* showing the enlargement of the terminal cells to form spores. *B*, stratum, partly in surface view. *D*, surface view of spores. *F* and *H*, some of the rows of cells enlarged. (*F* and *H* $\times 1400$, all other figures $\times 750$.)

There may perhaps be some doubt about the reference of this form to the genus *Pseudonocobyrsa* (Geitler⁽⁵⁾, p. 121) which is characterised by having ellipsoidal or spherical cells with rather thick and close-fitting mucilage envelopes and arranged in distinct, sometimes branched rows which are united to form a hemispherical attached stratum. In *P. fluminensis* there are no such obvious sheaths to the individual cells as in *P. lacustris* (Kirchn.) Geitler (*Oncobyrsa lacustris* Kirchn.), and the cells are of far smaller size; moreover, it is doubtful whether branching of the rows is at all common. The other species, *P. siderophila* (Naum.) Geitler (*Paracapsa siderophila* Nau-

mann⁽⁹⁾, p. 1) has spherical cells and the stratum is encrusted with ferric hydroxide. In neither of these species is anything known as to the method of reproduction and should a production of spores, as here described for *P. fluminensis*, prove to obtain in the other species, the systematic position of the genus would have to be reconsidered.

Another genus that may be taken into account is *Radaisia*, of which one species has been recorded by Sauvageau (14), p. 373) as occurring attached to stones in streams in France and Algiers. This form is little known, but in the marine species of *Radaisia* the construction seems to be definitely filamentous, with a basal stratum bearing dense upright threads terminating in sporangia (cf. Setchell and Gardner⁽¹⁵⁾, p. 45). The form here described is, however, not filamentous, although in other respects there is a good deal of superficial similarity. Attention may also be called to the resemblance between the strata of *P. fluminensis* and those of certain species of *Gloeothece*, such as *G. linearis* Naeg.

(c) *Chroococcopsis fluminensis* n. sp.

(Fig. 7)

This species is probably a common early coloniser of the rocks, but is not easy to recognise when overgrown by the other forms, the more as it may produce compact strata rather resembling those of *Chamaesiphon pseudo-polymorphus*. It is most easily studied in material from the swift water, where the other forms are not so strongly developed. The strata which it produces are typically composed of separate groups of chroococcoid cells with bright or deep blue contents which are ordinarily scarcely granular (Fig. 7 A, B, D, E). The cells of these groups occur in twos, fours, or larger aggregates (Fig. 7 D-I) which are surrounded by delicate colourless sheaths showing no trace of stratification. The groups of cells in the strata are of quite varied size and show all stages of subdivision (cf. Fig. 7 E). They are often densely aggregated and commonly occur in several layers, but the top layer consists of just the same separate groups as the others. In some strata, however, the individual cells are so tightly fitted among one another that they come to be polygonal and their mode of origin may then be obscured or even, except at an occasional point, difficult to detect (Fig. 7 J, K); even the sheaths may be unrecognisable (Fig. 7 J). In such compact strata, moreover, the cells may tend to become arranged along definite lines, so that a somewhat filamentous appearance results (Fig. 7 K). In

the denser strata this may lead even to the production of upright rows of cells (Fig. 7 C), although this is rare. The cells vary between 5 and 8 μ in diameter.

Occasional cells (Fig. 7 E) or groups of cells (Fig. 7 L) have finely granular contents. Among the latter, especially at the periphery of the strata, there are always to be found empty cells, with the membrane or sheath ruptured on one side. In a few cases such ruptured cells were found with the spherical undivided protoplast still inside the membrane (Fig. 7 L, at the lower edge), while occasionally a rounded naked protoplast with a thin investment of mucilage was

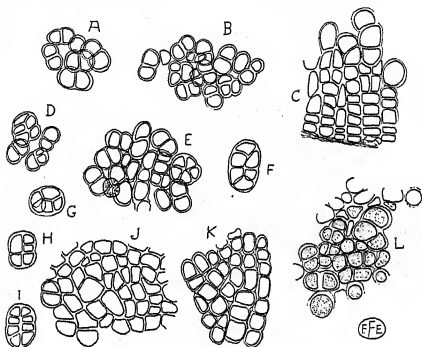


Fig. 7. *Chroococcopsis fluminensis* n. sp. A, B, D and E, typical young strata. C, old stratum showing a filamentous tendency. F-I, division stages. J, K, examples of compact strata. L, stratum showing reproduction. (All figures $\times 590$.)

found lying near one of the empty cells (Fig. 7 L, on the right). It seems therefore that this species propagates by means of these large gonidia (endospores) formed singly within the cells.

Chroococcopsis is a genus of Pleurocapsaceae established by Geitler (⁽⁴⁾, p. 342) for those forms which are not definitely filamentous. It has hitherto comprised only the single species, *C. gigantea* Geitler, which differs from *C. fluminensis* in the bigger cells, in the possession of a stratified sheath, in a less obvious filamentous tendency, and in reproducing by means of small endospores (gonidia) formed in large numbers in the cells. It is a genus which is rather

closely related to the Chroococcales, in fact in certain stages the British species would be hard to distinguish from some species of *Chroococcus*. Quite apart from the reproduction which is like that of the Pleurocapsaceae, however, the mode of occurrence of the genus is distinctive.

(f) *Oncobyrsa rivularis* Kütz. and *O. cesatiana* Rabenh.

(Fig. 8)

These two species of *Oncobyrsa* were best developed as epiphytes on the broad threads of *Cladophora glomerata*. Of *O. rivularis* Kütz. there were plenty of individuals showing only the basal layer in

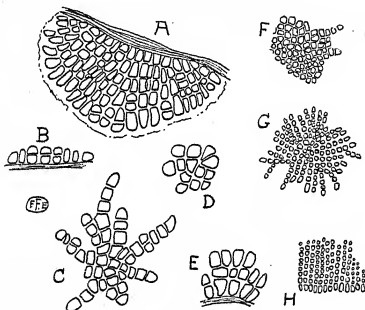


Fig. 8. A-E, *Oncobyrsa rivularis* Kütz. A, cushion in optical section; B, young stratum in section; C and D, basal layer, seen from the surface; E, somewhat older stratum in section. F-H, *O. cesatiana* Rabenh.; F and G, basal layer; H, stratum in section. (A-E $\times 520$; F-H $\times 900$.)

various stages of development (Fig. 8 C, D), as well as extensive mature, usually hemispherical, cushions with dense radiating rows of cells arising from the basal layer (Fig. 8 A). The latter usually shows a clear filamentous character, the branched threads radiating out from a central point and the branches generally terminating in a rather longer cell (Fig. 8 C) which only occasionally showed the bent form described and figured by Geitler. In some cases, however, the cells of the basal layer form a pseudo-parenchyma almost from the first, with little indication of the filamentous character (Fig. 8 D). The upright rows are produced by the division in a plane parallel to the substratum of the cells of the basal layer (Fig. 8 B); since all the

cells are involved compact cushions are produced. The cells of the upright rows are frequently grouped in pairs in a *Chroococcus*-like manner. Occasional divisions take place at right angles and thus a forking of the rows is brought about. Often the end cell of the row is rather larger than the others, but this is not always the case (cf. Fig. 8 A). It is questionable as to how far the growth of the upright rows is apical. A delicate layer of mucilage lies a very little way beyond the end cells of the rows. The cell contents were always bright blue and homogeneous, the cells 3.5–7 μ wide.

Geitler (4), p. 350) unites *O. cesatiana* Rabenh. with *O. rivularis* Kütz., stating that he has found the differences between the two species to be inconstant. In the material in the Devonshire streams, however, the two forms appear very distinct. The cells of *O. cesatiana* are very much smaller (diam. 1.5–2 μ) and paler coloured. The basal layer may, as in the other species, be clearly filamentous (Fig. 8 G) or pseudo-parenchymatous (Fig. 8 F). The strata have no definite shape and do not project as much as in *O. rivularis*. They are composed of densely placed, upright rows of rounded or slightly angular cells which appear to be unbranched and without an obvious mucilage envelope (Fig. 8 H). The lowest cell of each row forming the basal layer is elongated at right angles to the substratum¹.

(g) *Xenococcus chroococcoides* n. sp.

(Fig. 9 A–K)

This species has been observed only on *Cladophora*. Typically it forms fan-shaped groups of large pyriform cells which may or may not be subdivided and which are provided with a thick colourless stratified membrane (Fig. 9 F, K). The cell contents are a vivid blue and harbour a small number of dark coloured granules which are generally peripheral in position.

The groups originate from single cells by division. These cells are circular when seen from the surface (diam. 8.5–10 μ ; Fig. 9 A) and pear-shaped when seen from the side (long. 10–17 μ ; Fig. 9 G), sometimes a little curved (Fig. 9 H). Such cells undergo division along three planes. By division along the longitudinal axis they form groups which appear like those of a *Chroococcus* when seen from the surface (Fig. 9 B–E) and which initiate the fan-shaped groups when seen from the side (Fig. 9 J, K). By division transverse to the longi-

¹ Budde's ((2), p. 285, fig. 13) figures of "Antheridienstände" of *Hildenbrandia* look very much like a growth of *Oncobyrsa cesatiana* (cf. also Budde (3), p. 372).

tudinal axis they form the characteristic pairs of cells of which most of the pyriform units in the fan-shaped clusters consist (Fig. 9 *F, K*). In most cases it seems that this marks the finish, but a few larger aggregates have been found (Fig. 9 *I*), in which manifestly further division of the cells along the transverse axis had taken place.

This species is closely related to *X. kernerii* Hansg., but is distinguished by its larger cells, by the prominent stratification of their membrane, and by its very ill-defined filamentous tendency. According to Geitler (5), p. 135) *X. kernerii* usually develops upright threads from a basal pseudo-parenchyma which originates from the divisions of the chroococcoid groups.

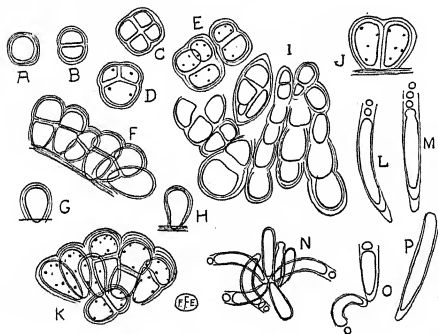


Fig. 9. *A-K*, *Xenococcus chroococcoides* n. sp. *A-E*, surface views of single cells and aggregates; *F* and *K*, typical groups in side view; *G* and *H*, single cells in side view; *I*, larger fan-shaped stratum; *J*, division stage in side view. *L-P*, *Chamaesiphon curvatus* Nordst.; *N*, a typical colony. (All figures $\times 600$.)

(h) *Chamaesiphon curvatus* Nordst.

(Fig. 9 *L-P*)

This occurred both as single individuals and as characteristic clusters (Fig. 9 *N*), often forming very dense strata, attached to *Cladophora*. The cells are often of considerable length ($14-68 \mu$ long; $4-6.5 \mu$ broad; Fig. 9 *L, M, P*) and provided with a close-fitting colourless sheath. The latter, in the larger individuals, was always thickened basally, so that the protoplast appeared to be withdrawn some little distance from the point of attachment (Fig. 9 *L, M*). This

basal thickening is quite hyaline and shows no stratification. Not uncommonly the lower end of the protoplast is somewhat attenuated, so that the outline of the whole is slightly pear-shaped. There is successive abstriction of gonidia at the apex (Fig. 9 *M*), the gonidia being in general slightly narrower than the protoplast from which they are cut off. While many of the solitary individuals were scarcely curved, some of those in the clusters always showed this feature very clearly.

(2) *Gongrosira fluminensis* n. sp.

(Fig. 10)

This species was mainly observed amid the thin growth found on rocks in the full torrent of the East Lyn, but there is very little doubt that it occurs more widely. In the fully developed condition it possesses a compact creeping basal portion attached to the rock surface and producing from practically every cell an upright thread, the latter in their entirety growing to approximately the same height (68–180 μ) and forming a very compact tuft (Fig. 10 *G*). It is, however, only in some cases that the projecting system attains to so considerable a development as is illustrated in Fig. 10 *G*; quite often the upright threads are quite short, composed of only three or four cells (Fig. 10 *M*), and this seems to be the commoner condition.

The cells of the basal stratum are in great part more or less rounded (diam. 8.5–10 μ) with thick irregularly stratified colourless walls (Fig. 10 *A*). Such strata may be very extensive and the figures only represent very small portions of them. Over considerable stretches the cells may be so closely fitted together as to form a compact pseudo-parenchyma (cf. Fig. 10 *D*), but at other points the filamentous character is clearly recognisable. The cells in the dense parts of the strata are often polygonal in outline. Many of the strata showed no upright growth at all and such form small bright green specks on the rocks. There is no calcification either of the creeping system or of the upright portion.

The latter consists of threads which scarcely branch at all in their lower portions, but towards the tips extensive branching occurs leading to the formation of numerous short branches, the majority of which consist of only three or four cells (Fig. 10 *G*). The branches arise from the upper ends of the cells, just below the septa (Fig. 10 *L*). The cells of the upright threads are rectangular or very slightly barrel-shaped, from one and a half to two times as long as broad (diam. 7 μ ; length 10–12 μ), and the walls are not nearly as strongly

thickened as in the basal stratum. There are one, two, or three pyrenoids in the cells, but the details of the chloroplasts could not be deciphered.

The end cells of the ultimate branchlets were always somewhat swollen (diam. $8-8.5\mu$), without however having any thicker walls than the others. Sometimes it was just the end cell that was enlarged, but far more commonly these swollen terminal structures were in pairs (Fig. 10 *F*), frequently with an oblique septum separating the

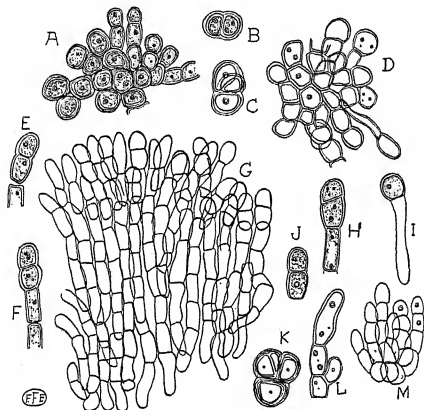


Fig. 10. *Gongrosira fluminensis* n. sp. A and D, parts of the basal stratum; B, C and K, akinetes, in part dividing; E and F, akinetes in course of detachment; G, fully developed upright system; H, apex of one of its branches, showing akinete formation; I, germinating akinete (?); J, detached akinete; L, apex of threads showing branching; M, young basal stratum with short few-celled upright branches. (G $\times 435$; all other figures $\times 600$.)

two cells of the pair (Fig. 10 *H*); there was always a pronounced constriction between the two. There is a great tendency for these enlarged cells to become detached from the threads bearing them. Many cases were found, like that shown in Fig 10 *E*, in which the septum between them and the underlying cell had split, although sometimes such splitting occurred lower down on the branch (Fig. 10 *F*), so that an ordinary cell became detached with the two swollen ones. Many detached pairs of cells were found in the material (Fig. 10 *J*).

Although there is no special accumulation of food reserves in these cells, it can hardly be doubted that they function as akinetes and serve for the propagation of the species. Many groups of cells were found, such as are shown in Fig. 10 C, K, in which subdivision was clearly taking place, and these cells already had the thick stratified walls of the basal stratum. Cases like that shown in Fig. 10 I may represent another stage in the germination of these akinetes, in which a colourless rhizoid-like prolongation is being formed. Such stages were not uncommon among the cells of the compact strata (cf. Fig. 10 D).

This species resembles *G. incrustans* (Reinsch) Schmidle (*Chlorotylum incrustans* Reinsch) in the densely arranged, little-branched upright threads, but the latter is encrusted with carbonate of lime. *G. leptotricha* Raineri (18, p. 23) also shows some resemblances. *G. scourfieldii* G. S. West (18), found in a rapid stream near Sidmouth, Devonshire, is calcified and all parts are richly branched.

(4) THE PHORMIDIUM COMMUNITY

Large sheets of *Phormidium* form a very conspicuous feature on the bigger boulders of most of the rapidly flowing streams. Walking on the footpath above the right bank of the East Lyn, these extensive sheets of a vivid blue-green or darker slate-blue colour are very striking. They are often a foot or more in diameter, frequently roughly circular, with an edge produced into many rounded lobes (Pl. V, phot. 6). They are best developed and occur in the largest numbers where the current is at its strongest, and are not nearly so common in the quieter parts, while where the water tends to be stagnant they are usually altogether wanting. For the most part they are completely submerged, such small portions of the sheets as project being frequently washed over, and it does not seem that they occur in parts of the stream where they would tend to become exposed to the air for longer periods. The principal species in the East Lyn is *Phormidium retzii* (Ag.) Gom.¹ (Fig. 3 J), which forms the bright blue sheets, but at many points this is more or less overgrown by *P. autumnale* (Ag.) Gom. (Fig. 3 K), which gives the sheets a darker slate-blue colour. In the slower-flowing Badgeworthy Water, the *Phormidium* community (here *P. autumnale*) is not nearly so prominent and is only to be met with at the points of most rapid flow. In the part of the River Heddon above Hunter's Inn *P. retzii* is very

¹ The majority of the trichomes have blunt, non-conical terminations, although occasional tips are conical. The trichomes are narrow and of astonishingly uniform width (4-4.5 μ) throughout the stratum.

conspicuous in places. No *Phormidium* community was noted in the UMBER.

Both in the East Lyn and the River Heddon it is very obvious that the *Phormidium* sheets favour the sides of the boulders that face towards the current, although they are not by any means confined to these places. In the former situations they must pick up a good deal of silt at times when the water is muddy, and the older strata do indeed harbour a quantity of fine matter. It is a striking fact that, although common enough in the *Chamaesiphon* community, *Phormidium foveolarum* with its much narrower threads plays no part at all in the *Phormidium* community, which in these streams at least is constituted solely by species with broader trichomes. Gomont ((6), p. 161) also, of the narrower species, records only *P. purpurascens* (Kütz.) Gom. and *P. valderianum* Gom. as occurring in streams¹. The reason for the scarcity of narrow forms is not apparent, unless it be that the tangled threads of the coarser species are alone able to withstand the force of the current without a disintegration of the stratum.

There can be little doubt that the *Chamaesiphon* community is often (or perhaps always?) the precursor of the *Phormidium* community. On the underside of the sheets of the latter *Chamaesiphon pseudo-polymorphus* is often found in extensive aggregates, the individuals of which even present quite a healthy appearance as though the overlying *Phormidium* stratum did them no harm. In other cases one finds underneath the *Phormidium* sheets brown detritus in which only few remains of algal growth can be detected. The succession would seem to be *Cocconeis*—*Chamaesiphon* community—*Phormidium retzii*—*Phormidium autumnale*. The surface of the strata is usually devoid of growth, although a few Diatoms (mainly *Cocconeis*) are sometimes to be found.

(5) CONCLUDING REMARKS

The above description will have shown clearly that blue-green algae are by far the most abundant constituents of the encrusting flora in these rapid streams. It is probable that they are of greater importance than the lichens, although the latter play a considerable rôle. It is a striking fact that, as in the cold lakes of the Antarctic and the hot waters of thermal springs, members of Myxophyceae are again the important pioneers in these, likewise extreme, conditions.

¹ *P. purpurascens* is also recorded by Watson ((17), p. 79) as occurring on rocks which are frequently submerged or kept moist by splashes or spray.

In the vast majority of cases green algae are conspicuously absent from the encrusting community, and *Gongrosira fluminensis* is the only one that can be said to be at all frequent. Its occurrence in the Devonshire streams is no doubt not an isolated case, since a somewhat similar form was found on the stones in a small tributary of the River Ammer on the Austrian frontier, although here only the parenchymatous basal stratum was properly developed, so that the species has not been ascertained. Moreover, various other species of *Gongrosira* have been recorded from streams. It may also be recalled (p. 178, footnote) that in a small stream in the Black Forest a species of *Coccomyxa* (probably *C. subglobosa* Pascher) was not uncommon in the *Chamaesiphon* community. Occasionally, too, filamentous green forms may hug the surface of the stones in such a way as almost to form a crust; thus, in the same Black Forest stream a species of *Stigeoclonium* formed a dense covering on many of the pebbles, whilst in the River Umler a *Cladophora* was found growing in the same way.

Hildenbrandia rivularis, though a frequent form, is not abundant and does not generally favour the same situations as the *Chamaesiphon* community. It has been met with in most of the Devonshire streams examined, and is usually, though not invariably, accompanied by *Lithoderma*. Diatoms, except for *Cocconeis placentula*, play no rôle in the encrusting communities dealt with in this paper.

There can be little doubt that the three types of encrusting algal communities distinguished in this paper are to be found in many fast-flowing waters, both in this country and abroad. One may, however, expect considerable variations in different kinds of streams. All of those so far mentioned have non-calcareous waters. In the Gorge du Chauderon above Montreux, where the water is definitely calcareous, none of the algal forms above described were encountered. Here the encrusting growth was very scanty, consisting of strata of *Schizothrix coriacea* (Kütz.) Gom. and an occasional brown fluffy growth of various Diatoms (*Cymbella helvetica* Kütz. var. *gracilis* Meister, *C. turgidula* Grun., *Gomphonema olivaceum* Kütz., etc.). It is not improbable that the character of the encrusting communities may be indicative of different types of water and especially that they may be an index of the purity of the water.

The diverse encrusting forms show a number of common features. All are of compact habit and hug the surface of the rock. There is a broad area of attachment and little surface exposed to the current, so that the latter cannot detach the alga from its substratum. The

vertical growth is dense, whether it be composed of numerous aggregated individuals (*Chamaesiphon* spp.) or of distinct upright threads (*Hildenbrandia*, *Lithoderma*, *Chamaesiphonopsis regularis*, *Pseudonobrysa fluminensis*, *Gongrosira fluminensis*). In practically all cases the outer surface is mucilaginous, a feature well seen in *Hildenbrandia* and specially marked in the species of *Phormidium*, so that the friction between the rapidly flowing water and the surface of the alga will be relatively slight. Propagation is probably in the main effected by detachment of cells (gonidia in the *Chamaesiphonales*, akinetes in *Gongrosira*, small fragments of the thallus in *Hildenbrandia*), i.e. by methods that are essentially vegetative. There must be an enormous wastage in the reproductive cells, although evidently many achieve a foothold. Presumably any small irregularity in the surface of a pebble will serve as a halting-place for the minute gonidia, etc., that are being swept along by the current and here they will probably cling by virtue of their mucilaginous surface. It is noticeable that practically all the members of the encrusting communities have small or very small cells. The absence of any marked accumulation of food reserves in the reproductive cells is noteworthy. There can be no doubt that the diverse encrusting forms exercise a marked erosive effect on the pebbles and boulders upon which they occur. Gentle scraping with a blunt instrument detaches usually not only the crusts, but also small particles of the substratum which are found firmly adhering to the underside of the algal material.

In conclusion it may be emphasised that the present report is based on observations made only during a limited period. It is desirable that they should be carried out also at other times of the year and, if possible, on other rapid streams (e.g. those of Wales and Scotland). If this account stimulates others to undertake such investigations it will have achieved one of its purposes. A great help in the study of the development of the different forms may be found in the exposure of suitably prepared glass slips at various points along the course of the stream (cf. Naumann (8)).

(6) DIAGNOSES OF THE NEW FORMS

The following are brief Latin diagnoses of the new forms described in the present paper:

Chamaesiphonopsis nov. gen. *Chamaesiphonacearum* (pp. 173-176, Fig. 3 A-I).

Thallus e strato basali et e filamentis erectis constans; pars basalis parenchymatica, substrato firme affixa, e cellulis polygonalibus arcte

coaltis aequalibus membranibus tenuibus composita; pars erecta e filis verticalibus vel radiantibus, dense aggregatis et valde regularibus, cellulis elongatis oblongis sine membrana composita. Propagatio per constrictionem gonidiorum modo *Chamaesiphonis* fit; fila erecta e gonidiis in situ germinatis oriuntur.

Chamaesiphonopsis regularis n. sp. (syn. *Xenococcus britannica* F. E. Fritsch; *Oncobyrsa britannica* F. E. Fritsch).

Stratis brunneis, macroscopicis, ad saxa in fluminibus affixis; cellulis oblongis, pallide aerugineis, raro rubris, cytoplasmate homogeneo vel parum granulato. Diam. cell. basal., $4.5-5\mu$; diam. cell. erect., $3.5-4.5\mu$; long. cell. erect., $10.5-14\mu$; diam. gonid., $3.5-4.5\mu$.

Chamaesiphon pseudo-polymorphus n. sp. (pp. 176-178, Fig. 4).

Stratis brunneis vel interdum rubro-fuscis, macroscopicis, ad saxa in fluminibus affixis, e cellulis numerosissimis, irregulariter et dense aggregatis constantibus; cellulis $6-8\mu$ latis, semper cum membrana (pseudovagina) distincta, formae diversae sed saepe brevibus ($9-10\mu$ longis), interdum tam latis quam longis, pallide aerugineis vel interdum rubris, cytoplasmate homogeneo vel cum granulato singulo; gonidiis saepe angustioribus quam cellulis vegetativis, plerumque hemisphaericis vel deplanatis, interdum sphaericis, saepe in series productis.

Chamaesiphon ferrugineus n. sp. (pp. 178-180, Fig. 5).

Stratis brunneis, macroscopicis, ad saxa in fluminibus affixis, e cellulis numerosissimis dense et plerumque irregulariter dispositis, interdum modo *Dinobryon* familias efficientibus, constantibus; stratis saepe e seriebus duabus cellularum compositis; cellulis pallide aerugineis, contentu homogeneo, cum pseudovagina distincta, cyathiformi, interdum stipitata, primo tenui et vix colorata, demum plus minus incrassata luteo-fusca vel brunnea, ferruginea, saepe fimbriata; gonidiis sphaericis, eadem magnitudine quam cellulis vegetativis, saepe singulis vel binis. Diam. cell. c. vag., $3.5-7\mu$; diam. cell. sine vag., $3.5-4.5\mu$; long. vag., $10-13\mu$.

Pseudoncobyrza fluminensis n. sp. (pp. 181-183, Fig. 6).

Stratis deplanatis, plerumque ad 70μ crassis, microscopicis, ad saxa in fluminibus affixis, e seriebus verticalibus cellularum parallelis numerosissimis, mucro hyalino vel luteo nidulantibus, constantibus; cellulis minutis, saepe oblongis polis rotundatis, interdum fere quadratis, semper in seriebus distinctis dispositis, nonnunquam cum vagina tenui hyalina, contentu pallide aerugineo saepe cum granulato singulo conspicuo; multiplicatio cellularum in series verticales per divisionem

transversam fit; sporis (?) terminalibus, singulis vel raro in seriebus brevibus, latoribus quam cellulis vegetativis, globosis vel leviter elongatis, rare pyriformibus, membrana distincta lutea vel fusca. Cell. veg., 2μ lat. et $3.5-5\mu$ long.; diam. spor., $2.8-4\mu$.

Chroococcopsis fluminensis n. sp. (pp. 183-185, Fig. 7).

Stratis microscopicis, ad saxa in fluminibus affixis, e familiis parvis numerosissimis compositis; familiis saepe distinctis, e cellulis 2, 4, vel pluribus, modo *Chroococci* dispositis et membranis tenuibus distinctis, constantibus, sed interdum arcte congestis cellulis polygonalibus, rare in filis brevibus dispositis membrana indistincta; cellulis plus minus laete aerugineis, contentu homogeneo; propagatio per expeditionem contentus totius cellularum quae granulas minutas continent fit. Diam. cell., $5-8\mu$.

Xenococcus chroococcoides n. sp. (pp. 186-187, Fig. 9 A-K).

Stratis microscopicis, flabelliformibus, ad *Cladophoram glomeratam* in fluminibus affixis; cellulis caeruleis, membrana crassa hyalina lamellata, contentus cum granulis paucis plerumque periphericis; cellulis singulis a vertice visis circularibus, a latere visis pyriformibus; post divisionem a vertice visis modo *Chroococci* dispositis, a latere visis saepe binis superpositis per divisionem transversam cellularum pyriformium ortis. Diam. cell. max., $8.5-10\mu$; long. cell., $10-17\mu$.

Gongrosira fluminensis n. sp. (pp. 188-190, Fig. 10).

Thallus minutus, ad saxa in fluminibus affixis, non calce induratus, e strato basali firme adnata et e filamentis erectis in caespites densos aggregatis constans; pars basalis e filis valde ramificatis, dense congestis (ita ut pseudoparenchyma cum cellulis plus minus rotundatis vel polygonalibus saepe oritur) composita; cellulis partis basalis membranarum crassis hyalinis irregulariter lamellatis; filis erectis in partem inferiorem vix ramificatis, in partem superiorem cum ramulis brevibus 3-4-cellularibus multis, cellulis rectangularibus vel leviter doliformibus, $1\frac{1}{2}$ -2plo longioribus quam latis, membranarum non incrassatis, pyrenoidibus 1-3 in utraque cellula; propagatio per disjunctionem cellularum terminalium singularum vel saepe binarum, paullo dilatatarum, membranarum non incrassatis fit. Diam. cell. basal., $8.5-10\mu$; diam. cell. erect., 7μ ; long. cell. erect., $10-12\mu$; diam. akinet., $8-8.5\mu$.

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DESCRIPTION OF PLATE¹

Phot. 1. Pebble from the East Lyn showing numerous crusts, in the main due to *Chamaesiphonopsis regularis* and *Chamaesiphon pseudo-polymorphus*.

Phot. 2. Pebble from the part of the River Heddon above Hunter's Inn showing crusts due to the red form of *Chamaesiphon pseudo-polymorphus*. The upper part of the pebble was covered by another. Note the elongation of the crusts in the direction of flow of the water.

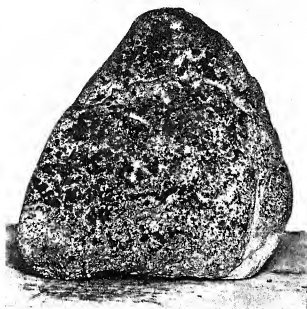
Phot. 3. Pebble from the River Umler, showing two thalli of *Hildenbrandia rivularis*; the remaining patches are due partly to *Lithoderma fluviatile* and partly to *Verrucaria submersa*.

Phot. 4. Pebble from the bed of Badgeworthy Water, showing numerous crusts due to *Chamaesiphon ferrugineus*.

Phot. 5. Pebble from the River Umler just above Coombe Martin. The upper patches are mainly formed by the thalli of *Lithoderma fluviatile*; in the lower part Lichens are frequent.

Phot. 6. Boulder from the East Lyn showing a stratum of *Phormidium retzii* Gom. (considerably reduced).

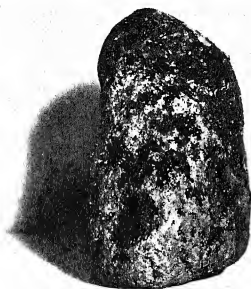
¹ The photographs on this plate are the work of Mr R. Cullen, Laboratory Attendant, Botanical Department, East London College.



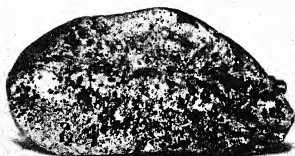
Phot. 1



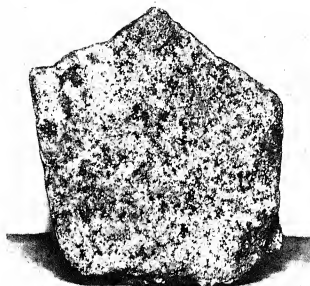
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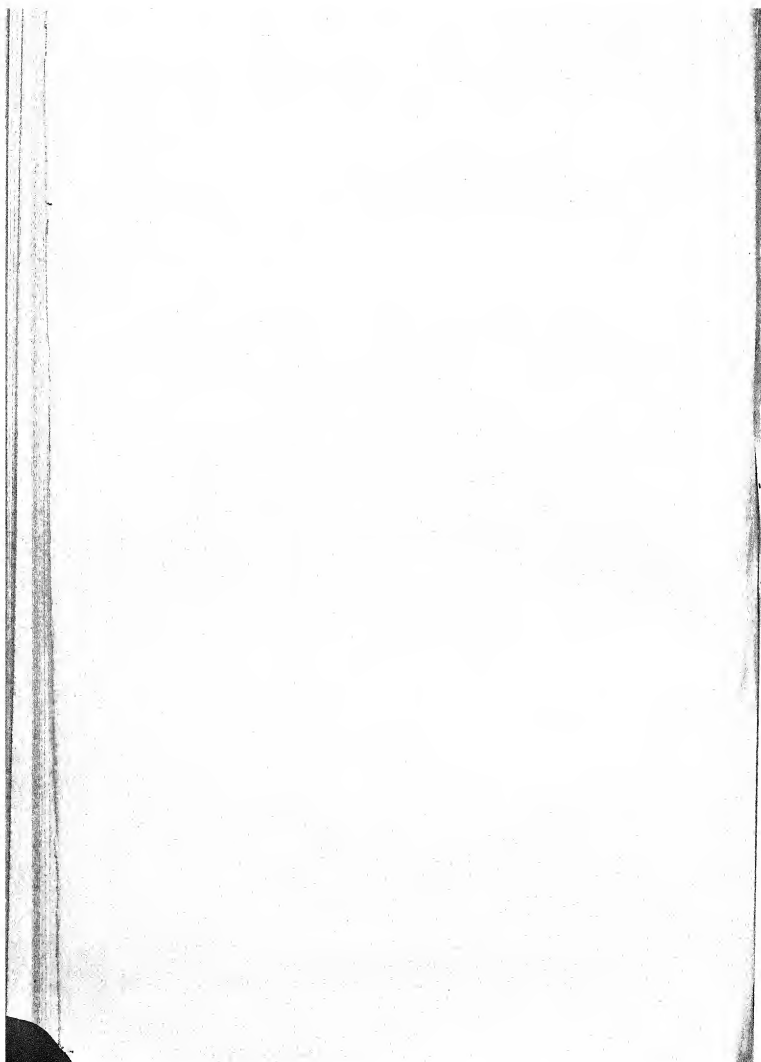


Phot. 5



Phot. 6

FRITSCH—ENCRUSTING STREAM ALGAE



THE BIOLOGY OF THE LIVING CHLOROPLAST

A CRITICAL ABSTRACT OF PROFESSOR LUBIMENKO'S
REVIEW OF RECENT RUSSIAN WORK

By J. H. PRIESTLEY

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INTRODUCTION

PROFESSOR V. LUBIMENKO has recently published in French (1,2,3)¹ a general account of a long series of experimental investigations upon the pigments of the chloroplast, in great part carried out under his direction, which have up till now been little known amongst English botanists because the earlier papers were either published in Russian or in somewhat inaccessible journals. This work has been in progress since the early years of the present century. Fortunately, however, its discussion at the present time may begin from the basis of facts as to the chemical nature of the pigments extracted from the green leaf with organic solvents. By the elaboration of a suitable technique, Willstätter and Stoll isolated and purified a number of these pigments and, thanks to the mastery of chemical methods possessed by these investigators, there is now general agreement as to their chemical nature. The attempts subsequently made by these workers to interpret the biological activity of the pigments showed that the basis of knowledge of the conditions at the photosynthetic centre was still not sufficient.

¹ Very full bibliographies will be found in these papers. In the following review no attempt is made to extend this list of citations, but the discussion is restricted to the data presented by Lubimenko. Where authors are cited without reference, therefore, the necessary reference will be found in these bibliographies.

The present statement of the new Russian contribution by Professor V. Lubimenko does not lead directly to a fuller understanding of the photosynthetic assimilation of carbon dioxide and the first production of organic substances in the plastid, but it does seem to throw quite a new light upon the conditions existing in the chloroplast, the centre of photosynthetic activity, and so deserves to be widely known amongst botanists.

In its French form, and with its full bibliographies, the work is very accessible. All that is attempted therefore in the present statement is to give some idea of its scope.

A critical account is not attempted, and if some of Professor Lubimenko's statements are challenged, it is rather to bring out the extraordinary interest of the problems raised, than to attempt to put aside his conclusions.

Big advances in many branches of botanical investigation may be expected when the botanist learns the discipline of the new instruments that the physicist and chemist make available for his use. Two outstanding examples could at the present moment be cited:

(1) The use made by Dr Sponsler, himself a botanist, of the X-ray method of crystal analysis to determine the fundamental structure of the cellulose wall. Reference to the brief paper recently published by W. Sponsler⁽⁴⁾ in which the results of the work are summarised for botanical readers, will show in what a new light problems of wall composition have now to be regarded, and that the "micellar" hypothesis in its old form is now finally disposed of.

(2) Similarly Lubimenko's work illustrates the power of the spectroscope and spectro-colorimeter, as tools in the hand of the biologist. It is fitting that their employment in this field should be in Russian hands in view of the prominence earlier given to optical methods in the investigation of photosynthesis by Timiriazeff⁽⁶⁾.

THE RELATION OF THE PLASTID TO THE CELL

Plastid inheritance and Plastid nutrition

Lubimenko begins by considering the old problem raised by Schimper as to the degree of individuality possessed by the chloroplast. Schimper argued that the chloroplast should be regarded as a separate organism maintaining its own independent life in the tissues of the higher plant and reproducing itself independently so that from generation to generation of plants the plastid was handed on by multiplication of pre-existing plastid.

For many of the algae, the plastid could be kept continuously in view throughout a life cycle which included a mobile swarmer. In the higher plant, the plastid, during the reproductive cycle of the plant, at least vanished from view and, for some time, the arguments of A. Meyer and many other cytologists seemed to have prevailed, and the chloroplast was regarded as forming anew in each individual which arose from the previous generation through sexual germ cells. Such a conclusion might necessitate the treatment of the chlorophyll apparatus of the lower plants, in which the chloroplast persisted through all reproductive stages, as a different type of physiological mechanism. Of recent years, however, the discovery of the mitochondrial apparatus has drawn attention to the possible significance of cytoplasmic organisation taken into the fertilisation machinery by the nucleated sex cells and the old controversy as to the permanence of the plastid throughout the complete life cycle of the higher plant is now being fought over again with a wealth of new cytological technique and terms. In view of the fact that as the plastid enters the germ cell or emerges from the cell of the apical meristem, it approaches to or emerges from the limits of microscopic vision, it is impossible to assert with confidence that it does not persist throughout the life cycle.

Plastid inheritance. With the development of genetics as a science, however, other points of view may be laid under contribution, and Baur pointed out that in at least some variegated forms, the inheritance of the chloroplast machinery can be interpreted in the terms of Mendelian segregation of certain pairs of unit characters. This would suggest to the geneticist that at least part of the machinery associated with chloroplast inheritance was in the nucleus in the germ cell. On the other hand, in the case of *Mirabilis Jalapa*, *Capsicum annum*, and *Pelargonium zonale* definite evidence that the chloroplast machinery is handed on only by one parent suggests that in these cases, plastid gives rise to plastid throughout the germinal tract from this one parent to the offspring.

Lubimenko, using quantitative spectro-colorimetric methods, compares the distribution of chlorophyll in the offspring obtained by crossing two pure lines of a species which differ very markedly in chlorophyll content. Out of fifteen crosses, he finds a chlorophyll distribution in the F_1 and succeeding generations, which can be interpreted on Mendelian lines in six cases; in the other nine cases, no such interpretation seems possible. Genetical analysis, therefore, suggests that both methods of chlorophyll inheritance may be

involved. Chloroplasts may persist as units throughout the life cycle, through the cytoplasm of the germ cell, but factors segregating on Mendelian lines in the nucleus may determine their behaviour during somatic development in succeeding generations.

Plastid nutrition. Lubimenko emphasises the importance of considering the chlorophyll content of the plastid in the light of the long series of cultural experiments now available in which the lower green plants have been maintained alive on artificial media.

Chodat, Dangeard and many others have thus established that the green colour of the algae has little connection with exposure to light, in which their behaviour agrees with many observations on Bryophyta, Pteridophyta and Gymnosperms, though in some cases chlorophyll develops more readily and rapidly in light. The relation of chlorophyll production to the nutrient substrate is a complex one. Lubimenko emphasises two points: (1) the best conditions for growth and for chlorophyll production are by no means closely correlated, and (2) many experiments suggest a certain antagonism between the requirements of the growing protoplasm and the growing plastid.

If the carbohydrate supply of the alga is increased for instance, increased growth of protoplasm may result with an increasing demand for nitrogen, which in a nitrogen deficient nutrient medium may be obtained by plastid disorganisation. On the other hand, if additional nitrogen is supplied, increased growth is accompanied by an increased production of green chloroplasts.

The carbohydrate requirements for protoplasmic increase and plastid increase seem to be far from identical. Chodat found with *Chlorella lacustris* that xylose and dulcitol slow up growth and favour chlorophyll production; on the other hand, galactose seems to be readily available either for growth or plastid production; arabinose seems of little utility to the plastids. Molliard and Matruchot, working with *Stichococcus bacillaris* found that it grew vigorously in glucose and laevulose, producing yellow colonies; saccharose, lactose and maltose gave slower growth but a good green colour; glycerol and mannitol, of secondary importance for growth, proved most favourable for the production of chlorophyll.

Molliard has shown that the same general type of experimental result can be obtained in experiments with flowering plants. Thus radishes with their roots in 2 per cent. glucose grow best, but with higher concentration of the sugar more chlorophyll is produced in the slower-growing plants. In this case, sugar applied to the cut base of the leaf has a different effect, causing a certain amount of

chlorophyll disorganisation, possibly because in experiments under these conditions too little nitrogen was supplied to the cells.

From the physiological standpoint then, there is a general correspondence between the behaviour of the chloroplast in the cell of both lower and higher plants, and from the same standpoint the disappearance of the plastid in the germ cell of the higher plant may be associated with the special nutrition of this cell. It is still an open question whether the plastids' invisibility in these cells may mean their complete disappearance, but the occasional appearance in genetical work of strains of seedlings which are completely devoid of chlorophyll and which therefore are not bred to maturity, certainly suggests that it may. The same thing seems to have happened in the case of saprophytic species of *Chlorella* and *Chlorotecium* and parasitic species of *Rhodochytrium*, etc.

If it should prove impossible to develop green pigment under cultural methods in parasitic or saprophytic forms of higher plants, the same argument would apply, but *Lathraea* of course has very well-developed leucoplasts, and in *Cuscuta*, in culture on organic media, Molliard has succeeded in developing chlorophyll. In the chlorotic areas of some variegated plants, the chloroplast organisation seems to be entirely absent. The nature of a rudimentary chlorophyll apparatus is, however, at present a very speculative question, and it might not be recognised; this problem will attain a little more precision later.

Whilst the chloroplast as such is normally quite invisible in the higher plant in the germ cell, after fertilisation, cell division and growth proceed with a rapidly changing balance of metabolites between embryo and ovule.

Schimper points out that in *Linum austriacum* chloroplasts are visible in the embryo at the eight-celled stage, and Lubimenko finds in a general spectroscopic study of the pigments of the developing embryo, that chlorophyll is present during development in 12 out of 110 families of Dicotyledons, Monocotyledons and Gymnosperms examined. Maturation is associated with another change in conditions of nutrition and in the majority of these cases the chlorophyll disappears at this stage. On germination chlorophyll usually rapidly appears again in the embryo, the amount being as a rule much less in seedlings grown in the dark.

ETIOLATION AND CHLOROPHYLL PRODUCTION

In seedlings of *Picea*, the amount of chlorophyll in those grown in light and dark is almost the same; in *Pinus* and *Larix*, the seedling in the light has more. *Picea* seedlings thus resemble the young sporelings of ferns and bryophytes which similarly develop chlorophyll in the dark and in this respect scarcely differ from algae in the behaviour of the plastid, but it must be remembered that the nutrition of these sporelings is frequently more closely similar to that of an alga in its more immediate dependence upon an outside habitat. Seedlings of Angiosperms, on the other hand, with the more complex system of nourishing the young embryo, are usually (though not entirely without exception) incapable of producing chlorophyll until they are in the light. These seedlings in the dark do contain small quantities of a green pigment, with a different absorption spectrum to chlorophyll, which Lubimenko and his co-workers termed chlorophyllogen, because in the presence of light it is converted into chlorophyll, a process which can be followed in the living etiolated leaf by observation with the Sorby Browning micro-spectroscope.

At about the same time (1909) that Lubimenko and Monteverde described chlorophyllogen, Liro (1908) and Issatchenko (1909) had argued that chlorophyll arose from a colourless mother substance—leucophyll—which is assumed to be present in etiolated plants. If the etiolated tissue dies in presence of water or alcohol, then this leucophyll gives rise to a green pigment—protochlorophyll, which differs from chlorophyll and is identical with the pigment extracted from etiolated leaves by Monteverde. But though this pigment is thus identical with Lubimenko and Monteverde's extracted pigment which will, therefore, be called protochlorophyll, it is not identical with the pigment, with corresponding absorption spectrum, detected by the micro-spectroscope in the living etiolated leaf, where it is seen to change into chlorophyll. Unfortunately, neither this more labile chlorophyllogen, nor the hypothetical leucophyll, have been obtained outside the living plant. If the etiolated plants are dried the chlorophyllogen persists and on exposure to light, spectro-measurements of the amount of protochlorophyll and chlorophyll in the extracts at different times show that it changes into chlorophyll, but the dried plants do not turn green, there is too little of the original chlorophyllogen there and no more is regenerated in the dry plant. The presence of chlorophyllogen can then only be deduced from the presence of protochlorophyll in the extracted pigments and from the observation of its absorption spectrum in the living plant.

That chlorophyllogen should thus defy extraction will seem very natural from Lubimenko's standpoint when his interpretation of the nature of the normal pigments of the plastid is put forward (p. 208).

Chlorophyllogen has never been present in etiolated Angiosperm seedlings in sufficient quantity to turn them green, but in the development of the inner coats of the seeds of certain genera of the Cucurbitaceae it accumulates in considerable amount, though in no other of the 110 families examined was this found to be the case.

During the early stages of the development of the fruits of *Luffa*, *Trichosanthes*, *Momordica*, *Cucurbita* and *Bryonopsis*, when the young seeds are illuminated strongly, chlorophyll accumulates in the testa, but later it tends to disappear and chlorophyllogen appears in its place. In the seeds of *Luffa* chlorophyll disappears completely so that on extraction these seeds give only protochlorophyll. As the seed matures, the chlorophyllogen in the living seed itself undergoes change, partly into protochlorophyll, partly into a greenish brown derivative with a different absorption spectrum, but in various neutral solvents this substance changes into protochlorophyll.

In the ripe seeds of *Cucurbita*, *Trichosanthes* and *Bryonopsis* considerable quantities of protochlorophyll may also be found in the testa; the presence of this substance in the seeds in this stage is understandable as the thick mass of pericarp surrounding the seeds completely excludes the light.

The etiolated Angiosperm contains always but small quantities of chlorophyllogen, but in the light of these facts, the influence of light upon the colour of the etiolated plant may also be very suggestively discussed. •

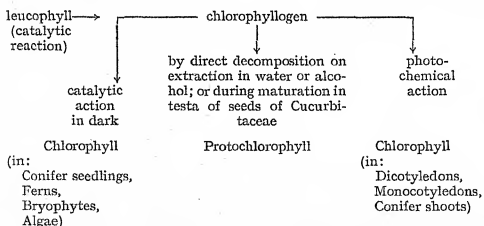
Evidently, given suitable conditions of nutrition, light is not necessary for chlorophyll formation, but it may facilitate the process either (1) directly, because it accelerates certain stages of the process as the change from chlorophyllogen or protochlorophyll to chlorophyll, or (2) indirectly, because it affects cell or tissue permeability and thus the nutrition of cell and plastid. In the seedlings of some Gymnosperms, as *Pinus* and *Larix*, nutrition without light provides but inefficiently for chlorophyll production, and Schmidt has demonstrated in *Pinus Jeffreyi* that the internal conditions determining the production of chlorophyll in the embryo are localised in the endosperm.

The isolated embryo growing in the dark is colourless, but if a piece of endosperm is added, its colour changes rapidly to green. This may simply mean that the necessary conditions of nutrition

have so far only been supplied by the endosperm; on the other hand, Lubimenko and Schmidt interpret this as evidence that a necessary enzymic factor is in this case located in the endosperm and that the change from leucophyll to green protochlorophyll or chlorophylligen is catalysed by this enzyme. But if this is so, then in the Angiosperm as a rule, some other internal factor limits the development of the green colour in the dark, a factor which can be overcome completely by exposure to light. The quantity of light necessary is greater, however, than is required to increase tissue permeability to an extent that enables the apical meristem to be nourished. Thus, with very low light quantities supplied through occasional brief exposures to weak light intensities, leaf and shoot production may proceed whilst chlorophyll production remains completely suppressed. In the conifer shoots also, unlike the seedlings, the nutrition of the shoot meristem as it pushes in the dark, never permits chlorophyll production.

In these shoots, then, as in the Angiosperm seedling, some factor would seem to be lacking for chlorophyll production which is supplied by exposure to light, but which in conifer seedlings is supplied by the endosperm in the dark and in ferns and the lower green plants seems usually to be present in the cell provided the external nutrition is well balanced and adequate.

Following Lubimenko, these conclusions may be expressed schematically:



In this scheme, there is as yet little experimental evidence for the existence of leucophyll or chlorophylligen. Leucophyll is assumed by Liro and Issatchenko and is supposed, when the cells die on extraction, to change into protochlorophyll. Lubimenko and Monteverde recognise chlorophylligen by its absorption spectrum (that of protochlorophyll) in the living etiolated leaf, where it can be seen

in light to change into chlorophyll. On extraction, chlorophylligen is assumed always to give protochlorophyll. There is never more than a small amount of chlorophylligen present in the etiolated leaf; it is therefore assumed that it is continuously formed from a colourless leucophyll. As the dead etiolated plant never gives rise to more than a very small amount of protochlorophyll it would seem reasonable to assume that this arises from the small amount of chlorophylligen present and that after death no more chlorophylligen is regenerated from the mother substance—the leucophyll.

The developing testa of the cucurbit seeds shows that an accumulation of chlorophylligen in the plastid is possible (though it is not clear from the evidence in these papers that the pigment in the testa actually regenerates chlorophyll, and is therefore chlorophylligen and not protochlorophyll), in the lower plants and conifer seedlings probably it is freely formed and as readily transformed into chlorophyll. Thus in the seedlings of *Thuja* and *Larix*, grown in the dark, where chlorophyll production lags under these conditions, protochlorophyll was recognised in the extracted pigments so that this stage is recognised in this type of plastid where the chlorophylligen can be converted into chlorophyll in the dark. In the Angiosperm seedling not only is the change from chlorophylligen to chlorophyll dependent on the light, but evidently the production of chlorophylligen is slight in the dark. This directs attention to the catalytic changes involved in the production of leucophyll or its conversion into chlorophylligen.

The very high temperature coefficient shown by chlorophyll production in etiolated seedlings at low temperatures

$$\begin{aligned} (Q_{10} &= 8-10 \text{ between } 5^{\circ}\text{C. and } 15^{\circ}\text{C.}), \\ (- &= 2.5-2.3 \text{ ,, } 18^{\circ}\text{C. and } 28^{\circ}\text{C.}), \end{aligned}$$

suggests that chlorophyll production in such seedlings is determined rather by enzyme reactions than by photochemical reactions, and Lubimenko suggests that these reactions are concerned with the production of leucophyll.

The special problem has seemed worth this detailed analysis because the whole trend of Lubimenko's work is to suggest that experimental resources are by no means exhausted in connection with it. So long as the potentiality to develop chlorophyll is there, the right nutritional conditions may cause its production even in the dark. There is, therefore, every reason to hope that at least chlorophylligen, if not chlorophyll, may be freely produced in such etiolated

seedlings and the consideration of such problems cannot but be of value in connection with many practical problems as to chlorosis induced by disease, cultural conditions, etc.

THE CHLOROPLAST IN CULTURE

Yet another line of experimental investigation is suggested by Lubimenko's conception of the relation of the plastid to the cell. Such a plastid may be independent of this cell, except as to supplies of inorganic food, and Schimper's early standpoint that it was a separate organic unit thus justified. From this standpoint, it might be possible to isolate the plastid from the cell and maintain it in its activities upon a suitable nutrient medium. Experiments to this end have not been lacking and Lubimenko refers to some early efforts of Famintzin (1866). Up till now, no one has succeeded in maintaining isolated chloroplasts alive and growing and multiplying, but Lubimenko has had some striking results by a very surprising method. He was led to try to release the plastids from the cell contents by allowing the tissues to undergo a natural fermentation.

Under these conditions, the fresh leaf tissue being sliced up in water in which it is left in a dense green mass, the nuclei and protoplasm undergo a type of autolytic digestion, the walls, with the exception of lignified and suberised layers, are gradually digested, but the chloroplasts remain green and healthy looking even after 14 days in such a putrefying mass.

From such mother liquor, chloroplasts were then transferred to various solid and liquid nutrient media. The usual glucose media did not prove very satisfactory. For the chloroplasts of the tobacco plant, the following media proved most satisfactory: (1) 1-5 per cent. glycerol and 0.2 per cent. asparagin; (2) 2-5 per cent. glucose and 0.2 per cent. asparagin; (3) the liquid obtained by filtering the putrefying mass of sliced tobacco leaves in water after a previous boiling. In such media, the chloroplasts remained green and apparently unaltered after nine months, but they were not seen to multiply and it is impossible to say they were alive. In the early stages of the fermentation process the plastids lose any starch they may contain, and none was subsequently ever seen to reappear in them. At least, however, another experimental technique is suggested which may lead ultimately to knowledge of value as to the biology of the living chloroplast.

THE SINGLE PIGMENT OF THE CHLOROPLAST

If there was one point that seemed clear after the work of Willstätter and Stoll, it was that in the great majority of green plants four pigments were present in the plastid, two chlorophylls *a* and *b*, and two yellow pigments, carotin and xanthophyll. These pigments could be separated by their different solubilities, and were chemically distinct, chlorophyll "*a*" differing from "*b*," and carotin from xanthophyll, by the equivalent of a molecule of oxygen. Whilst these conclusions as to *extracted pigments* must remain substantially unaffected, an outline of Lubimenko's arguments must now be presented, together with an indication of the supporting experimental evidence, which leads, on the contrary, to the conclusion that in every chloroplast there is but one pigment and that differs in different plants, possibly even from species to species.

In the first place Lubimenko affirms, upon the evidence of the absorption spectra, that the chlorophylls are not always identical. He distinguishes four groups of chlorophylls. From a small group of plants, *Urtica dioica*, *Plantago major*, *Lamium album*, *Anthriscus sylvestris*, and *Aegopodium Podagraria*, chlorophylls were obtained with eight definite absorption bands.

In a second and larger group, *Hedera Helix* may be cited, the seventh band was absent; the intensity and position of the seven remaining bands vary considerably in the group. A third group, of which *Prunus Laurocerasus* is a member, have the first and second bands united into one continuous band, whilst in another group of plants, of which *Uva Lactuca* is an example, there are only five bands, because the first is combined with the second, whilst the third is missing. Presumably, the chemical differences indicated by these spectroscopic differences are slight and indicate only subsidiary isomeric changes in the molecule; in each plant we may expect from Willstätter and Stoll's work, that chlorophyll *a* and *b* are present and distinct from one another by the equivalent of a molecule of oxygen.

Lubimenko compares these various chlorophyll spectra with that of the living leaf, and in no case are they identical. This point has already been discussed by Willstätter and by Stern and others, and the suggestion made that the difference arises from the fact that in the leaf the chlorophyll is dispersed in colloidal solution whilst after extraction the absorption spectrum is usually determined with the pigment in molecular solution. Lubimenko is unable to agree, however, that the suspension of the pure pigments in water, or any other

colloidal solution of the pure pigments tested, has an absorption spectrum identical with that given by the same pigments before extraction, whilst Stern gives reasons for thinking that the chlorophyll in the plastid is in true solution. In making the comparison, full allowance is made for the bands of the associated yellow pigment which, however, are really too weak to disturb the direct comparison. Lubimenko has prepared an aqueous suspension from the leaf, by fine grinding in water, which has an absorption spectrum identical with that of the leaf, and in the case of *Aspidistra elatior* and certain other plants, this colloidal solution of pigment can be filtered off from the leaf debris and obtained as a very fine stable green liquid.

This is not, of course, the usual pure solution of the two extracted green pigments, chlorophyll *a* and *b*; it must contain both the yellow pigments and also protein; but it is more equivalent spectroscopically to the normal pigment of the plastid.

Lubimenko then discusses the surprising difference in yield of the yellow pigments obtained with the usual organic solvents from the fresh leaf. The original concentration of this solvent depends upon the amount added and the water content of the leaf, and with variations in the original concentration there are very marked variations in the amounts of yellow pigments obtained when these are examined quantitatively by spectro-colorimetric methods. (But these yellow pigments are extraordinarily stable and nothing that occurs during the process of extraction is likely to destroy them. If they are not obtained, therefore, in greater quantity under some conditions, it must be because they are not there, and that means that under certain conditions of extraction the yellow pigment is not there to the same extent, presumably because it still forms part of a larger molecule.

Then, again, the green suspension obtained from the filtered, crushed leaves of *Aspidistra* is relatively stable to acids, whilst the molecular solutions of the extracted chlorophylls are, as is well known, exceedingly sensitive to weak acids. If this fine suspension is treated with any of the usual organic solvents, or is boiled, or in fact is given any treatment liable to denature the protein in colloidal solution, then the stability of the green pigment to acids disappears. Lubimenko draws the natural conclusion that the protein, chlorophyll *a* and *b*, and carotin and xanthophyll, are all held in one complex molecule, the pigment molecule.

This state of affairs elucidates, on slightly different lines to Willstätter's original explanation, the old puzzle as to the inability

of dry acetone to extract chlorophyll from air dry leaf powder. In such air dry material the pigment complex is still in being, and when the acetone is added, in the absence of any water, it is unable to precipitate the protein. In the presence of a little water this process takes place and the usual extraction of the green and yellow pigments follows.

The case for regarding the pigment of the living chloroplast as contained in one complex molecule seems a very strong one, the spectroscopic comparison of the living leaf of one species with another suggests further that these complex molecules may differ in some cases from species to species. The very wide range of yellow pigments obtained on extraction from plastids, as described in a later section, points in the same direction.

THE AMOUNT OF CHLOROPHYLL IN CHLOROPLAST AND LEAF

The spectro-colorimetric method, as developed by Lubimenko and Monteverde, permits a fairly rapid measurement of the quantities of chlorophyll present in any individual leaf. It is, therefore, readily possible to make comparisons between different plants, whether different species or not, or even between the different leaves upon the same plant.

Sun and shade plants

Lubimenko has thus carried out a systematic examination of the average weight of chlorophyll per kilogram of fresh weight of leaves to be found in the leaves of many species from habitats lying in different latitudes. The general conclusion emerges that the average quantity of chlorophyll present increases as we move from the Polar regions towards the Equator. This increase is due in the main to an increase, in the warmer climates, of the number of species of a "shade" type which are able to utilise a weaker light in photosynthesis as pointed out already by Willstätter. In one and the same species the quantity of chlorophyll it contains may vary in the same way with latitude, and in one and the same plant it varies in different leaves in accordance with their degree of exposure. Adaptation to shade is shown by a reduction in thickness of lamina followed by a diminution in number of chloroplasts, together with an increase in their individual dimensions and chlorophyll content. It is these characteristics of shade plants which result usually in a high chlorophyll content per unit of fresh weight. From this standpoint Lubimenko distinguishes three classes of plants as the result of the quantitative comparisons:

1. Shade plants, rich in chlorophyll, not plastic in structure and physiology and unable to endure too much insolation.

2. Sun plants, equally non-plastic in character and relatively poor in chlorophyll.

3. Plastic plants, containing a very variable quantity of pigment and able to endure wide range of habitats so far as light is concerned.

Diffuse light is much more deficient in red rays than direct sunlight and preliminary observations suggest that typical shade plants utilise the short wave-length end of the spectrum more efficiently in photosynthesis than the sun plants.

Marine algae of all colours are usually regarded as specially adapted to the peculiar light conditions existing at varying depths of the sea. However this may be, Lubimenko's first results are very surprising and suggest that a new field of research is opening here. Compared with the more highly organised plants, all these algae are very deficient in chlorophyll, even compared with another submerged plant such as *Zostera marina*. The chlorophyll content shows no correlation with depth, *Laurentia Coronopus*, a surface form, having a minimal quantity of chlorophyll, whilst *Phyllophora rubens*, a deep-water form, is relatively well supplied with this pigment.

VARIATION IN CHLOROPHYLL CONTENT

The variation in chlorophyll content in the leaves of a single plant, and its apparent connection with degree of exposure to light, suggests that light, which we already know to have a direct effect upon the original chlorophyll production at least in the higher plants, may also exert a direct influence upon the chlorophyll content of the older leaf. It can be shown experimentally with Angiosperm seedlings that a brief exposure to bright sunlight retards the subsequent rate of change to the normal green colour in diffuse light.

For each plant there appears to be an optimal light intensity, below which a maximum content of chlorophyll is not developed, and above which it is either not developed or is subsequently lost in further metabolic changes. This optimum is not a fixed constant of the species, however, but at least within limits will gradually vary, rising with continual exposure to a higher light intensity. That strong light can bring about a destruction of chlorophyll is a recognised experience, illustrated by the bleaching of shade plants in too strong light, and by the frequent change of chloroplasts to chromotaphores in foliar and floral organs in light under various conditions. Fluctuations of light intensity of this order of magnitude will result in

changes in sugar concentration, and Palladin has already shown with etiolated plants that chlorophyll production is favoured by a certain range of concentrations, though inhibited by concentrations higher than 10 per cent. Sugar accumulation would also follow not only upon a higher light intensity, but on a longer duration of the light period, and Lubimenko's work leads him also into the field of photo-periodism so vigorously cultivated of late years, since the experiments of Garner and Allard.

Photo-periodism

Lubimenko indicates that a more extensive statement of the problem raised by his photo-period experiments will follow. His preliminary generalisations, upon experiments carried out with natural daylight, artificially shortened by shading some of the plants for definite periods, may be summarised as follows.

Amongst the plants experimented upon three groups may be distinguished:

1. Plants of tropical origin, which show a maximum production of dry weight in periodic lighting with an optimum light period.
2. Plants of arctic origin, which are ill-adapted to periodic lighting. Certain of these plants, e.g. *Papaver nudicaule*, die rapidly if the day is shorter than six hours.
3. Plants of temperate regions which show an adaptation to a photo-period more like that of tropical plants.

The analogy with the classification of sun, shade and plastic plants will at once strike the reader.

In all this comparative biology of the green leaf, some clue is needed to the factors, internal and external, leading to greater loss of chlorophyll when light is too strong, too long-continued, or, as a result, the sugar accumulation too high. Many observers have shown that with some plants at least, high sugar concentrations are associated with lower photosynthetic efficiency.

Photosynthesis and respiration

Lubimenko finds the internal controlling factor to be the energy of respiration in the neighbourhood of the photosynthetic centre, the plastid. Many items of experimental evidence point this way, but the present writer's impression is that at present, too many separate lines of experimental evidence are forced into alignment with this particular interpretation, with the result that some experimental data are given a significance they are as yet unable to support.

It has long been known that the etiolated seedling did not turn green in the absence of oxygen. Liro has shown that the transformation of chlorophyllogen into chlorophyll takes place without the presence of oxygen, so that the oxygen must be required either for the production of leucophyll or of chlorophyllogen from leucophyll. Willstätter and Stoll showed that photosynthesis would not take place in complete absence of oxygen, and carried out some very suggestive experiments upon peroxide formation in chlorophyll extracts. Spoehr has shown carbon assimilation to increase with respiration and suggests that part of the energy of respiration may be utilised in the process of photosynthesis. Lubimenko finds such a view in complete accordance with his experimental results and as the result of a long series of comparative determinations concludes that tissues actively engaged in photosynthesis are also characterised by high oxidase activity. These experiments are not pursued in any detail, the quantitative method used to determine oxidases, depending upon the colour reaction with guaiacum resin, is not capable of great accuracy, and the "anti-oxidase," the reducing action of which Lubimenko finds can be suppressed by adding formalin, requires reconsideration in the light of Szent-Györgyi's recent paper(5).

These various lines of evidence suggest therefore that oxygen may be necessary for chlorophyll production and for photosynthesis. Lubimenko concludes that respiration is necessary for photosynthesis to proceed. In the process oxygen is utilised in oxidising sugars and in this fact may lie the significance of minimal quantities of sugar for the production of chlorophyll in various nutrition experiments, etc. Such sugar may also be necessary for photosynthetic efficiency and when the green leaf, after a certain period in the dark, fails to increase in dry weight this may mean that during the dark period all the sugars of the leaf have been oxidised.

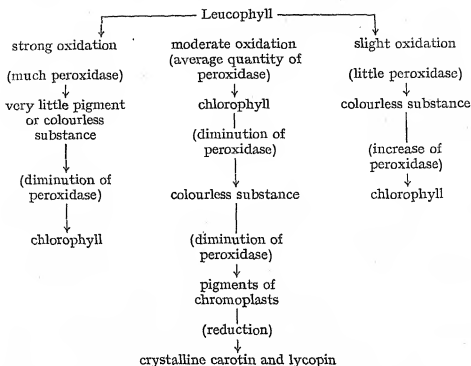
Lubimenko regards the arctic type of plant as so effective in removing its sugar that it can continue photosynthesis in uninterrupted 24 hour periods of illumination, whilst, if the dark periods are too long it is so starved that it dies.

On the other hand, if sugar accumulates too much, photosynthetic efficiency is cut down, possibly because the sugar concentration partially inhibits the normal oxidase activity. The tropical or temperate plant represents a plant in which with too prolonged illumination sugar accumulation then impedes oxidase activity, and therefore subsequently the resumption of photosynthetic activity if

the intervening period of darkness is not long enough. This is one of many possible alternative explanations of the photo-periodic phenomena shown by many plants, and may prove of value in elucidating some of these complex experimental problems. But any assumption that dry weight accumulation, in these experiments, is primarily determined by the rate of photosynthetic activity in the plastids, is far from proven.

Lubimenko has still to deal with the question of chlorophyll disappearance when the light is too strong or too long continued. He suggests that this may also be an oxidation process and cites the independent observation of Woods and Breslavetz to the effect that oxidases are more active in the chlorotic portion of variegated leaves. This does not seem very definite evidence that this oxidase concentration is causally associated with the absence of chlorophyll in these regions, but using crushed up leaf material in water, Lubimenko shows that the subsequent disappearance of chlorophyll from these crushed materials is due to an oxidation which is accelerated by light and good aeration, and which proceeds much more slowly after the enzymes in such crushed tissues are destroyed by boiling. Lubimenko also suggests that the reason that the epidermis of the tomato and of *Capparis spinosa* is free from chlorophyll is to be found in the great quantity of oxidising enzymes he finds in these cells. This relation of chlorophyll to oxygen and to oxidising enzymes and to their substrate sugar seems a very delicately balanced mechanism, especially in view of the various ways in which it may be influenced by light. As a result it is always possible to make any experimental result fit into the general scheme and at the same time difficult to devise the crucial experiment which finally establishes or destroys any suggested link in the chain of causation. With this comment, this section may be terminated by the general scheme which Lubimenko puts forward for the possible interplay of photosynthetic pigments and oxidation systems. (See over page.)

As an indirect line of evidence connecting respiration with photosynthesis, Lubimenko cites a number of interesting experiments in which the dry weight of seedlings is compared after the original leaf surface has been mutilated and reduced. But the argument is here too indirect. Apparently, first of all, the amount of leaf removed (in one case one or more leaflets of a lupine) is regarded as a measure of the injury, and then a proportion assumed between the degree of injury thus measured and the increase in respiratory activity resulting from wounding.



Thus the dry weight is regarded as an index of photosynthetic activity. In the regeneration following such wound experiments it is a well-known fact that the new leaf surface pushes out with increased vigour in proportion to the extent that, as a result of mutilation, the root system now preponderates over the shoot. Under such conditions, dry weight determinations are probably much more closely connected with the rate of increase of leaf area than with the relative efficiency of unit area. This appears to hold in Lubimenko's experiments from the figures given, and they are therefore not considered further here.

THE YELLOW AND ORANGE PIGMENTS OF CHLOROPLAST AND CHROMOPLAST

The spectroscopic method permits a very rapid survey of these pigments which is not possible so long as it is to be followed by detailed chemical examination. As a result Lubimenko has passed a very wide series of these yellow pigments in review, and drawn some very interesting conclusions, though so long as these rest upon this one line of experimental evidence alone some of them must be regarded as speculative, especially as the methods of separation of the pigments, thus controlled, do not guarantee that absolutely pure substances are being compared.

Lubimenko first finds, as Willstätter and Stoll had done, that in the developing leaf, at first the quantities of yellow and green pigments increase in about the same proportion. A fact which, in the light of the earlier experiments described, may be regarded as further evidence of the increase of one single complex chloroplast pigment. In the older leaves he finds on the other hand, that whilst all pigments increase in amount to a maximum in adult leaves, diminishing subsequently in ageing leaves before they fall, throughout life the proportion of yellow pigments to green tends to diminish. The assumption of autumnal colours seems often to be associated with the replacement of both the normal green and yellow pigments by different types.

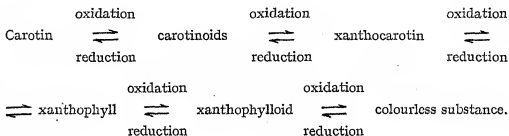
The absorption spectra of the carotins and xanthophylls from the normal chloroplasts of different plants showed considerable variation. This led Lubimenko to a much more extensive study of this series of pigments, for which he turned to the chromoplasts in which they are stored in greater quantity. The basis of the experimental method was a system of fractional extraction of the fresh material. Utilising the water in the living material, extraction was first carried out with cold ethyl alcohol, of concentration of from 85 per cent. to 90 per cent. Extraction with cold 96 per cent. alcohol followed, then with the same solvent boiling, then with cold petrol ether, then boiling. Thus five extracts were obtained. All were evaporated in darkness, the precipitates controlled under the microscope and, if from alcohol solutions, washed with petrol ether, if from petrol ether solutions washed with alcohol; the washings being evaporated down in a similar manner. Thus a series of precipitates were obtained, often crystalline, which were compared spectroscopically, but, to judge from the present papers, conclusions as to the chemical nature of the series of substances thus obtained are based simply on Willstätter and Stoll's earlier work and the observed tendencies of absorption bands.

The general basis of the above method is, of course, the fact that carotin is insoluble in alcohol and soluble in petrol ether, whilst xanthophyll is soluble in alcohol and insoluble in petrol ether. Carotin contains no oxygen, xanthophyll the equivalent of one molecule per molecule of hydrocarbon, and in the transition from carotin to xanthophyll the absorption bands shift towards the violet end of the spectrum. In the course of this work some allied compounds were found with different solubilities, xanthocarotins, soluble in both petrol ether and (slowly) in alcohol, but relatively insoluble

in formic acid; and xanthophylls soluble in all three of these solvents.

Of these the xanthophylls shifted the absorption spectra even further towards the violet end than xanthophyll, and this seems to be the only ground given for assuming that they contain more oxygen than xanthophyll.

Lubimenko, therefore, draws up the following scheme of the relationships of these substances:



Similarly the more orange pigments give a series of the same type between lycopin, the isomer of carotin, and rhodoxanthine, an isomer of xanthophyll.

These lycopins, through series (III and IV) of lycopinoids may change into carotin; similarly through other series of lycopinoids (I and II) they are transformed into rhodoxanthine and this to xanthophyll.

The Russian investigators, on spectroscopic grounds and solubilities, have thus listed a long series of substances, some of them obtained crystalline, which must await further examination at the hands of the organic chemists. They have also described one substance, distinct from these, which they think may, with the aid of protochlorophyll, partly bridge the wide gap between carotin and chlorophyll.

This substance, buxine, is formed in the leaves of *Buxus* when exposed to direct insolation on the seashore in the Crimea. All the chlorophyll in the leaves then disappears and the red compound is found instead. It dissolves in carbon bisulphide, petrol ether, boiling alcohol, etc., but differs from the carotins, which are stable in presence of concentrated hydrochloric acid, because it gives a greenish brown colour under treatment by this acid. Its absorption spectrum by its complexity gives it a place intermediate between chlorophyll and the normal yellow and orange pigments, whilst protochlorophyll on the other hand has a relatively simple chlorophyll spectrum. This may be so, but on the other hand it is difficult to visualise a very

direct chemical transition between chlorophyll containing the nitrogen linkages of the pyrrol nuclei and the complex cyclic hydrocarbon which must form the basis of carotin.

Lubimenko appears to tend towards this further assumption on the basis of these isolated spectroscopic data, that transition compounds between carotins and chlorophylls are to be looked for and thus the whole of the great range of widely diverse pigments derived from one common generative substance. But chemically, any common basis between the pyrrol linkages of the chlorophyll molecule and the complex cyclic hydrocarbons of the carotin series seem impossible to visualise. Nor do they seem necessary on the facts presented. This common generative substance, which is still hypothetical, must on the evidence presented contain a protein molecule. It will, therefore, already be so complicated and large a molecule that it may readily include the matrix of two great series of colour-bearing compounds, the chlorophylls and the carotins. There appears to be no case where the green plastid contains chlorophyll to the complete exclusion of the carotins, but where, in the chromoplasts of the flower or fruit, the carotin series are formed to the complete exclusion of chlorophylls, it is a simpler assumption to make that the chlorophyll generating matrix has disappeared, disorganised, from the complex molecule, than that it has been transformed chemically through a complex series of changes from chlorophyll basis to carotin basis.

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INJECTION EXPERIMENTS ON PLUM TREES IN RELATION TO *STEREUM PURPUREUM* AND SILVER-LEAF DISEASE

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INTRODUCTION

IN connection with investigations on Silver-leaf disease Brooks and Moore(1) pointed out in 1926 that certain pathological symptoms developed in the leaves when stems of plum trees were injected with the culture fluid in which *Stereum purpureum* had been grown, but from which all traces of the living fungus had been removed. These symptoms involved a yellowish mottling or a wilting and browning of the leaves immediately above, or otherwise near, the places of injection. In those early experiments, carried out from 1923-25, the injections never resulted in silvering of the foliage. During the last three years many injection experiments, chiefly of a somewhat modified type, have been performed, and these have demonstrated conclusively that silvering of the foliage as well as other pathological symptoms can be induced by injecting plum trees with non-living extracts of *Stereum purpureum* in the used culture fluid or by injecting them alone with the culture fluid in which the fungus has been grown.

The mode of injection of the stems of these trees was that described by Brooks and Moore(1), which has been found entirely satisfactory. In the most recent experiments a fairly large reservoir has been attached to the intake tube in order to obviate the need for frequent replenishment of the injection fluid. During the last three years these experiments have been commenced in March, i.e. at the time when the buds of the plum trees (in an unheated greenhouse) are beginning to expand, in order to bring the developing leaves under the influence of the injection fluid throughout their growth. As the rate of intake of liquid at any one injection hole falls rapidly after about ten days, a series of injections were made at intervals, vertically above one another, so that the expanding buds in one particular region of the tree were constantly affected by the injection fluid. As the injection holes ceased to be used they were closed with sealing wax.

PREPARATION OF FLUIDS USED FOR INJECTION

As experience had shown that the most profuse mycelial growth was obtained by growing the fungus on sterilised plum twigs standing in an asparagin-glucose-starch-mineral salt medium this kind of culture was invariably used in the experiments. Growth of the fungus was started from spores on a solid medium in Petri dishes, and when it was certain that a pure culture had been obtained, small portions of mycelium were transferred to the upper extremities of the plum twigs in the culture flasks. The mycelium grew down the twigs into the nutrient fluid, on the surface of which a dense mycelial mat was formed. The reason for inoculating first the upper extremities of the twigs rather than the culture fluid direct was to obviate the risk of contamination. If mould fungi obtained access to the flasks during inoculation their colonies could be seen either on the twigs or in the fluid medium before the mycelium of *Stereum purpureum* reached the latter, and such flasks could be discarded. Without this precaution the purity of the cultures could not be guaranteed.

As silvering did not result in the 1923-25 experiments by using for injection the filtered fluid in which the fungus had grown, it was decided in later experiments to obtain an extract of the fungus for injection in the following manner: a portion of the mycelial mat together with some of the used culture fluid was removed from the flask, placed in a mortar containing sterilised sand, pounded up vigorously, and then filtered through ordinary filter paper into sterile tubes. The fluid thus obtained, diluted if required with sterile water, was used immediately for injection. Previous experience had shown that it was impossible to keep the injection fluids entirely free from bacterial contamination under the conditions obtaining in these experiments, so filtering through a Berkefeld bougie, as in the earlier experiments, was dispensed with. In using ordinary filter paper precautions had to be taken to use mycelium entirely free from the spores as the latter were found to pass through such paper. Spores of *S. purpureum* are rarely produced under the conditions of culture described above, and then only in very old cultures, so the risk of spores being present was entirely avoided by using young mycelium. The fluid obtained in this way for injection was quite clear and usually remained so for several days, but if it became cloudy owing to bacterial contamination it was discarded forthwith. Toluol was sometimes added to this kind of injection fluid in order to inhibit the development of bacteria, but, as will be pointed out later, this made no difference to the results obtained on injection.

The culture fluid in which the fungus had grown was also used for injection after filtration.

Portions of these fluids were boiled for five minutes before being used for injection, and control experiments were carried out, in which sterile distilled water, distilled water + toluol, and the culture medium, were used for injection.

RESULTS OF INJECTION EXPERIMENTS

(A) *With fluid obtained after pounding up young mycelium in the liquid medium in which it had grown*

Eight young Victoria plum trees were injected from about the end of March onwards. About 4-7 days after injection the tips of the sepals and petals of many flowers immediately above the injection hole became brown, and the browning often extended to the base of these organs. Shortly afterwards, the tips or margins of young leaves in the same region also became brown. These symptoms appeared somewhat later in higher parts of the trees, especially on the same side as the injection holes, and, to a limited extent and more erratically, below the lowest place of injection. At the top of these trees the affected leaves were irregularly distributed. A little later still, some of the leaves with brown tips developed a number of small, irregular yellow spots in the lamina, which fell away after a time, leaving holes. The brown tips or margins of the leaves also fell off. Subsequently, many of the leaves on each of the eight trees became silvered. On examination, these silvered leaves showed the same histological symptoms as those characteristic of silvered plum leaves in nature.

Two other Victoria plum trees were injected with this fluid to which a few drops of toluol had been added. The effects on the flowers and foliage were the same as when toluol was omitted.

Some of the fluid obtained after pounding up the mycelium was diluted with sterile water to one-quarter strength and was then used for injecting seven other Victoria plum trees. The results were identical with those obtained by injecting with the full-strength fluid. With two young Czar plum trees, however, injected with this diluted fluid, the only pathological effect was silvering of the leaves, there being no browning of the flowers or leaf-tips.

(B) *With fluid as in (A), but boiled for five minutes before injection*

Two young Victoria plum trees were injected with this fluid, but although browning of the flowers and leaf-tips occurred the leaves did not become silvered.

Two other Victoria trees injected with this fluid, diluted to one-quarter the normal strength, gave the same result. A Czar plum tree injected with the same diluted fluid showed no pathological symptoms at all.

(C) With the fluid in which the young mycelium had been growing

Of four young Victoria plum trees which were injected with this fluid all showed browning of the flowers and leaf-tips, but only three became silvered subsequently. A young Czar plum tree treated in the same way showed no browning of the flowers or leaf-tips, but became silvered. These results differ from those obtained with the same kind of fluid used in the 1923-25 injections described by Brooks and Moore(1), when there was no silvering of the foliage.

(D) With fluid as in (C), but boiled for five minutes before injection

One young Victoria plum tree, injected with this fluid, showed browning of the flowers and leaf-tips, but no silvering, and a Czar plum tree, similarly injected, exhibited no pathological symptoms of any kind.

(E) With fluid obtained after pounding up old mycelium in the liquid medium in which it had grown

The injection fluid was prepared in the same way as "fluid (A)," but the mycelium used for this purpose was two years old, the culture flasks having remained free from contamination. No sporophores had developed in these cultures, but, for the reasons stated earlier in this paper, there was no guarantee that spores were not present in the injection fluid, although this was unlikely.

Two young Czar plum trees were injected with this fluid diluted to one-quarter of the normal strength; both became silvered, but only one of these trees showed browning of any of the leaf-tips.

Another Czar plum tree, injected with some of this fluid which had been boiled for five minutes, showed no pathological symptoms.

(F) With the fluid in which old mycelium had been growing

This fluid was that in which the mycelium referred to in connection with "fluid (E)" had been growing for two years. Two young Czar plum trees were injected; both became silvered, and one showed browning of the tips of some small leaves before silvering was apparent.

(G) *Control experiments*

Other young Victoria and Czar plum trees were injected with the fresh culture fluid, with distilled water, and with distilled water + toluol, but these injections had no adverse effect upon the trees.

These results afford convincing proof that the same effect, viz. silvering of the foliage, can be produced in plum trees by injecting them with an extract of the mycelium in the used culture fluid, devoid of all traces of the living fungus, as by inoculation with *Stereum purpureum*. In the more recent experiments silvering has been induced also by injecting trees with the fluid in which the fungus has been growing for some time, hence the substance which directly or indirectly causes silvering is present in this fluid. Presumably, this substance passes out from the mycelium into the fluid. The minimum time between the commencement of injection and the onset of silvering was 15 days, a period considerably shorter than that which elapses between inoculation with the living fungus and the appearance of silvering of the foliage. The shortness of the period between injection and the development of silvering affords indirect proof that the pathological symptoms were not induced by chance inclusion of portions of the living fungus. It is to be expected that in these injection fluids there would be a greater concentration of the substance or substances which lead to silvering than would be produced during the early stages of development of mycelium in the tissues.

With Victoria plum trees the onset of silvering was preceded by browning of the flowers and leaf-tips as well as by other pathological symptoms occasionally. In this connection it is noteworthy that silvered Victoria trees, which contain the living fungus, sometimes show these symptoms also during the unfolding of the buds, although in the writers' experience these effects are never so striking as in the injected trees. With injected Czar plum trees, however, these additional pathological effects were apparent only on two occasions.

When the above fluids were boiled for five minutes before injection no silvering resulted, although with Victoria plum trees browning of the flowers and leaf-tips was still evident. It appears therefore that one of the substances associated with the causation of pathological symptoms in silvered trees is thermo-stable.

The fact that silvering was induced by injecting fluid in which the fungus had grown for two years indicates that the agent which causes silvering is probably stable for long periods at ordinary temperatures.

Many parasitic organisms are now known to secrete substances of a toxic nature which cause symptoms of disease in parts of the plant remote from the actual seat of the parasite. For instance, Hutchinson⁽²⁾ demonstrated in 1913 that the wilting symptoms of tobacco plants attacked by *Bacterium solanacearum* are induced by a toxin secreted by this organism, and, more recently, Brandes⁽³⁾, Bewley⁽⁴⁾ and Dowson^(5,6) have shown that yellowing and wilting of the foliage of plants affected by species of *Fusarium* and *Verticillium*¹ are caused by the secretion of toxic substances, which are carried up to the leaves in the transpiration stream. The behaviour in this respect of *Stereum purpureum* in the tissues of woody plants is essentially the same, but the pathological symptoms induced by the secretion of toxic substances by this fungus appear to be more diverse in character than those caused by the other organisms mentioned.

EXAMINATION OF THE WOODY PARTS OF SOME OF THE INJECTED TREES

Two of the trees injected with "fluid (A)" were cut up for examination four months after injection had begun, i.e. a considerable time after silvering had become apparent. The xylem on the side of the injection holes was found to be discoloured, owing to the accumulation of gum-like substances, just as in trees invaded by *Stereum purpureum*, the discoloration extending for several inches above and below the injection holes. The discoloration, however, did not pass into the small lateral branches or into the top of the tree, both of which bore silvered leaves. Sections of the discoloured wood showed hyphae occasionally; this is not surprising in view of the lapse of time since the injections had been started and of the impossibility of carrying out these experiments under completely aseptic conditions. The fungi occasionally found in the discoloured wood were isolated in culture in the usual way, but *S. purpureum* was never obtained. It was concluded therefore that "fluid (A)" was capable of producing the same brown discoloration of the wood as is caused by the living mycelium.

Two other trees injected with "fluid (B)" (i.e. "fluid (A)" which had been boiled for five minutes) were examined in the same way. Although these trees exhibited only browning of the flowers and leaf-tips the amount and distribution of the discoloured wood in the main stem was essentially the same as in the trees injected with

¹ The organism described by Dowson was referred to by him as a species of *Cephalosporium*, but it is now known to be a species of *Verticillium*.

"fluid (A)." It would appear therefore that though boiling had destroyed the agent which causes silvering of the foliage, the high temperature had not affected the capacity of the fluid to cause extensive gum-formation in the wood. The hyphae occasionally found in the discoloured wood of these trees were not those of *S. purpureum*.

With trees injected with water or with the fluid used for growing the fungus gum-formation occurred only in the immediate vicinity of the injection holes.

CONDITION OF THE TREES IN THE YEAR FOLLOWING INJECTION

All the trees which became silvered or showed other pathological symptoms when injected exhibited normal flowers and foliage in the following year. This is a further indication that the fluids used for injections were devoid of living *S. purpureum*, as in all probability some of these trees at any rate would have been silvered again in the following year if *S. purpureum* had been inadvertently introduced into them.

SUMMARY

(1) Silvering of the foliage and other pathological symptoms, such as browning of the flowers and leaf-tips, are induced in plum trees by injecting their stems with a filtered, non-living extract of *Stereum purpureum* in the culture fluid in which the fungus has grown.

(2) The same effects are produced by using for injection only the culture fluid in which the fungus has grown for some time.

(3) Boiling of these fluids for five minutes before using them for injection inhibits silvering of the foliage but does not prevent the development of other pathological symptoms.

(4) These fluids, whether boiled or unboiled before injection, induce an extensive brown discoloration of the wood in the vicinity of the injection holes owing to the formation of gum-like substances. This effect on the wood is similar to that caused by the growth of *Stereum purpureum* in it.

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ILLUSTRATIONS OF CARPEL POLYMORPHISM

IV

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(With 91 figures in the text)

IN an earlier account¹ I have suggested that the typical papilionaceous ovary is constructed of two carpels, and further, that the two genera *Arachis* and *Scorpiurus* are exceptional and differ from the type form in having an ovary of several (± 10) carpels. In the present account it is proposed to discuss in greater detail the evidence on which this interpretation is based.

Examination of a large number of genera shows that the whole residual vascular cylinder in the Leguminosae is continued directly into the gynoeceum. That is to say, the gynoeceum not only appears to, but actually does, represent the termination of the axis. In no case were discarded vascular elements seen in the pith, such as were observed to occur in *Prunus* among the Rosaceae. It follows that if we adhere to the orthodox view that in the Leguminosae (those Mimosoideae which possess more than one style excepted²) the gynoeceum is constructed of a single carpellary leaf, we must be prepared to accept the corollary that a foliar member may not merely have the appearance of being terminal (as in the case where, through having a nearly circular exsertion, it simply tops the axis as a hat crowns the wearer's head), but may be genuinely terminal in the strict sense, arising through the continued activity of cells which are actually apical.

It will be obvious that the zygomorphic symmetry of the perianth in the Caesalpinoideae and Papilionatae and the often more or less excentric position of the ovary introduce a disturbing effect which is absent, or only slightly marked, in the Mimosoideae. It will therefore be preferable to deal first with types belonging to the last-named section.

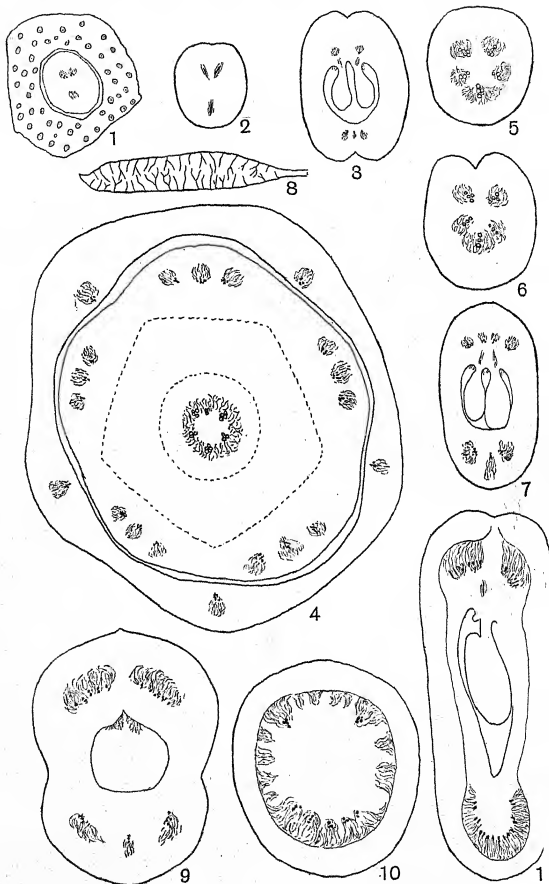
MIMOSOIDEAE

Flower actinomorphic, gynoeceum central.

Acacia suaveolens Willd. (Figs. 1-3). The ovary being central becomes disjoined from the flower wall on all sides simultaneously,

¹ See "Carpel Polymorphism, I," *Ann. Bot.* **39**, 142, and Figs. 39-49. 1925.

² *Affonsea*, *Archidendron*, *Hansemannia*.



Figs. 1-11

and a cross-section shows at first an even circular outline (Fig. 1). The vascular elements for the gynoeceum consist of a single front bundle and twin bundles towards the back. [*A. longifolia* shows twin bundles front and back.] After the gynoeceum has become free these bundles turn out to the periphery, the latter diverging as they go (Fig. 2). The increase in the anterior-posterior diameter now begins to exceed that in the transverse direction. Also with the out-turning of the twin bundles the contour line comes to show a bulge on the two corresponding radii, thereby giving rise to a depression in the back median line. In other words a posterior (ventral) furrow comes into being, not through a process of infolding, but owing to greater development on the radii of the two postero-lateral bundles than on the mid-line between them. The anterior strand, which also turned out towards the periphery at the same time as these two bundles, shortly gives off a pair of lateral branches. These branches develop more vigorously than the centre bundle and this difference is again

Figs. 1-11. Mimosoideae and Caesalpinoideae. All from transverse sections taken at successively higher levels except 8. 1-3. *Acacia suaveolens* Willd. 1. The ovary stipe surrounded by the androeceum from which it has just become disjoined, showing an even circular outline. 2. The free stipe. The single anterior and twin posterior bundles are turning outwards to the periphery, the latter diverging as they go, thereby leading to the appearance of a ventral furrow. 3. The ovary with ovules and placental hairs. In the front, a small median strand with a pair of more vigorous laterals. At the back, twin fertile cords with their placental strands directed inwards. In accord with this vascular arrangement the outer contour shows a dorsal as well as a ventral furrow. 4-8. *Albizia lophantha* Benth. 4. The flower base. To the outside the five sepals and five petals. Within the perianth the, as yet, undifferentiated androeceum belt. In the centre the vascular tissue for the gynoeceum, consisting of six strands with xylem, the posterior strand being less developed than the other five. 5. The ovary stipe immediately after disjunction from the androeceum ring. The weak posterior vascular strand has now come to an end, but the effect of its disappearance is not yet reflected in the contour line, which is still circular. 6. The same after the appearance of the ventral furrow which follows upon the disappearance of the posterior vascular strand. 7. The ovary. The placental strands which are given off laterally from the twin posterior cords stand near the middle line; the ventral outline has now in consequence become convex. 8. An unripe fruit showing the development of a system of lateral veins from both anterior and posterior cords. 9. *Inga pulcherrima* Cerv. The ovary (middle region) showing the bi-symmetrical, hour-glass contour assumed as the vascular elements become concentrated at the anterior and posterior poles. 10, 11. *Bauhinia yunnanensis* Franch. 10. The ovary stipe showing an even circular contour. The vascular ring is surrounded by endodermal and pericyclic layers. Xylem elements are confined to the anterior sector and two postero-lateral bundles. 11. The ovary after rupture of the endodermal and pericyclic layers in the middle line at the back, with consequent appearance of a ventral furrow. [Figs. 1-7 and 9, 10 same magnification, 11 less highly magnified, 8 nat. size.]

followed by an alteration in the contour line, for having previously been evenly convex in front it now shows an indentation precisely similar to the posterior one (Fig. 3). So bi-symmetrical, indeed, does the outline become that in cross-section it is not possible, without the clue provided by the vascular anatomy, to tell which is the anterior, and which the posterior face.

We thus see that the existence of a furrow in the mid-line at the back in the leguminous ovary is not necessarily evidence that the furrow is the line of junction of the edges of a folded carpel, for in *Acacia* there is a furrow both back and front which clearly owes its origin to differences in the rate of extension on different radii, resulting from the particular distribution of the vascular tissue.

Albizzia lophantha Benth. (Figs. 4-8). This type illustrates the exceptional condition in which the vascular strands supplying the androecium do not turn outwards but continue their original upward course. Delimitation of the vascular tissue for the gynoecium is therefore brought about by the giving off centrally, and turning inwards towards the pith, of the requisite elements. These elements become reconstituted into an inner continuous ring in which six xylem strands occur distributed round the circle, one being much weaker than the others, showing sometimes only a single xylem vessel (Fig. 4). As the gynoecium becomes free from the flower wall it exhibits in cross-section an even circular outline, and it is seen that the weak xylem strand which was situated in the mid-line at the back has now died out altogether (Fig. 5). The same change in the posterior contour ensues (Fig. 6) as in *Acacia*, but here the furrow is shortly obliterated and then replaced by a slight ridge. This altered contour, as before, is in direct relation with the vascular development. To right and left of the back mid-line are two of the original five stronger bundles with xylem. These two bundles supply the placenta. The other three persist as the anterior mid-vein with a lateral on either side. The placental branches arising from the two posterior bundles are not given off *towards the centre* as in *Acacia* (Fig. 3), but *laterally*, so that they are close to the middle line and to the periphery (Fig. 7), hence the increased development on the posterior median radius which leads first to a flattening out, and then to a conversion of the concave into a convex outline. The anterior contour remains evenly convex, for in *Albizzia* the centre bundle is no less well developed than the lateral pair (Fig. 7). In the developing fruit lateral veins take their rise both from the anterior and the posterior cord, the two systems reaching about equal development (Fig. 8), as is the case in other

genera belonging to this section and in certain Caesalpinoideae, notably *Haematoxylon campechianum*.

Inga pulcherrima Cerv. (Fig. 9). The ovary, which here first becomes disjoined from the flower wall along the two sides, is oval in cross-section from the outset. Before separation is yet complete the residual vascular ring, till now entire, becomes interrupted laterally so as to constitute anterior and posterior sectors, which gradually become contracted towards the two poles. The xylem in the posterior sector is disposed in two extended arcs. The same arrangement comes about in the anterior arc but here there is in addition a separate bundle standing in the middle line. In accord with this disposition a posterior median furrow appears as soon as the posterior face becomes free, whereas the anterior contour line remains evenly convex. As in *Albizia* the ventral outline soon flattens out and then presently projects (Fig. 9). As the loculus makes its appearance the ovary becomes constricted in the lateral plane, appearing hourglass-shaped in cross-section—yet one more feature harmonising with the bi-symmetrical concentration of the vascular tissue (at the two poles) and the bi-carpellary construction of which this disposition is an expression.

In *Inga* (Fig. 9), as is the case also in *Acacia* (Fig. 3) and *Albizia* (Fig. 7), numerous hairs are borne on the placentae beside the ovules. It is of some interest that in *Inga* similar hairs arise also in the middle line in front at the level at which the loculus is about to be closed by conducting tissue. It is thus clear that the presence of hairs on the placentae does not here necessarily indicate the junction line of fused carpellary edges.

CAESALPINIOIDEAE

Flower zygomorphic, gynoecium generally more or less excentric.

Bauhinia yunnanensis Franch. (Figs. 10, 11). The residual vascular tissue here continues in the form of a closed ring bounded by a distinct bundle sheath and pericyclic layer throughout the length of the stipe, which possesses in cross-section an even outline, circular at first (Fig. 10), becoming oval later (Fig. 11). The front sector, extending perhaps round a quarter of the circumference, shows several separate xylem strands, one of which stands in the middle line. Two other smaller arcs situated towards the back, on either side of the middle line and at some distance from it, also show well-marked xylem elements. But in the back median sector and along the two sides no xylem vessels are differentiated, the vascular tissue consisting wholly of phloem. As the stipe passes into the ovary

proper it becomes flattened laterally, the vascular ring becomes interrupted at the back, and coincidentally a ventral furrow makes its appearance. In due course the loculus is formed and the vascular elements are withdrawn to the two poles, the bundle sheath and pericycle persisting as a ring interrupted only in the posterior median line (Fig. 11). Finally the whole amount of vascular tissue in front becomes consolidated into one mass with a continuous cap of fibres, while at the back are the twin arcs which give off the placental strands from their inner face. The presence at the ovary base in the posterior sector of additional vascular elements bridging the gap between these twin fertile bundles (Fig. 10) recalls the case of *Prunus*, where the same feature is well marked¹. Here, as there, the existence of this intervening vascular tissue is difficult to reconcile with the orthodox view that only one carpel is present and that the twin fertile bundles are its *marginal* veins. It can hardly be doubted that both here and in *Prunus* this intervening sector is the remnant, now undifferentiated and without xylem, of an almost vanished mid-bundle, the counterpart of the well-developed mid-bundle present in the sterile front cord. We find even stronger evidence for this view in certain other Caesalpinioideae (e.g. *Cassia*, see below) and in some Papilionatae (e.g. *Colutea*, see p. 245) where xylem elements are to be found in this position as well as phloem. But such cases are exceptional. In these sections of the family, possibly as the result of the particular form of zygomorphic symmetry which they exhibit, the posterior median bundle, which in the mimosoid genus *Albizzia* survives in such form as to leave no doubt as to its significance, has in many types undergone degeneration, culminating, in the extreme case, in its complete suppression.

Cassia laevigata Willd. (Figs. 12-21), *C. corymbosa* Lam. (Figs. 22-29). The very unequal development of the members of the androecium in this genus makes it necessary to take into account the manner of origin of the vascular supply of the gynoecium at an earlier stage than in the preceding cases. In the species investigated the two stamens superposed upon the keel petals have extremely massive filaments. The four standing in front of the antero-lateral sepals and wing petals are less stout, the one in front of the anterior sepal less stout still. All these seven produce full-sized anthers, while the three posterior members superposed upon the postero-lateral sepals and "standard" petal are short and slender, with poorly developed anthers. As soon as the vascular elements for each filament are

¹ See "Carpel Polymorphism, II," *Ann. Bot.* 41, 591 and elsewhere. 1927.

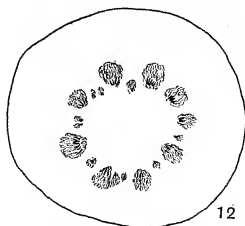
delimited they become disposed in a ring (Figs. 14-17). In the case of the stouter filaments these rings consist of a large number of strands surrounding a considerable area of parenchyma. This most unusual feature, which has not been met with in any other family so far examined, is to be seen in other caesalpinoid genera (e.g. *Brownea*, Figs. 39-41, and *Schotia*). After this whole vascular complex has passed out into the filament the ring becomes interrupted at one point (Fig. 20). The resulting horseshoe pattern is continued to the top of the filament where the anther lobes are attached (Fig. 21). After the emergence of perianth and stamen cords the residual vascular tissue again forms a ring of numerous xylem-forming bundles, the one in the mid-line in front causing a slight projection in the ring outline, in cross-section, owing to its having been carried outwards for a short distance conjoined with the trunk cord serving the anterior sepal and stamen (Fig. 15). A small bundle with xylem stands also in the mid-line at the back (Fig. 16). In *C. laevigata* this median bundle is shortly resolved into two fine strands from which the xylem has already disappeared at the level at which the ovary stipe becomes disjoined from the flower wall, only an arc of phloem bridging the space between two strongly developed bundles occurring one on either side of the middle line (Fig. 18). This intervening phloem also shortly comes to an end leaving a large gap between these paired bundles (Fig. 19). In *C. corymbosa* this median posterior bundle is still clearly seen in the ovary stipe even after it has become free (Fig. 22).

The disjoined *Cassia* stipe, like that of *Bauhinia*, shows an even contour line and the vascular ring is delimited by a bundle sheath and pericyclic layer (Figs. 18, 22, 23). The xylem elements are now aggregated into a central bundle and a pair of laterals in the front sector, and corresponding twin bundles with (in *C. corymbosa*), or without (in *C. laevigata*), a middle bundle in the back sector. In the former case the configuration at the one pole is the counterpart of that at the other (Fig. 22), but here, too, the posterior median xylem bundle shortly disappears (Fig. 23). The phloem elements in this position quickly follow suit. At the same time the endodermal and pericyclic layers become interrupted here, and also right and left in the lateral plane. A corresponding break occurs in the phloem belt extending along the sides of the stipe and connecting the two polar systems. As the back-to-front diameter increases, pericycle and bundle sheath are no longer to be traced in these non-vascular sectors. At this stage the characteristic bulge in the contour line appears over

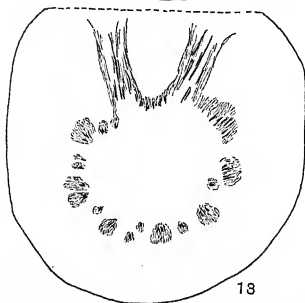
each of the two postero-lateral bundles leaving a depression in the middle line where vascular tissue is now lacking (Figs. 24, 25). This ventral furrow is presently obliterated (Fig. 26) and the outline becomes convex (Figs. 27, 28) in the same manner and for the same reason as described above in *Albizzia* (p. 228 and Fig. 7). Simultaneously the free ends of the crushed and ruptured pericycle and bundle sheath become pushed out towards the periphery (Figs. 26, 27). At the ovary apex, where a relatively large posterior sector again becomes devoid of vascular elements owing to the coming to an end of the two postero-lateral bundles, the furrow appears afresh (Fig. 29).

Saraca indica L., *Brownea coccinea* Jacq., *B. Crawfordii* W. Wats., *Schotia speciosa* Jacq. In these types the unequal development of

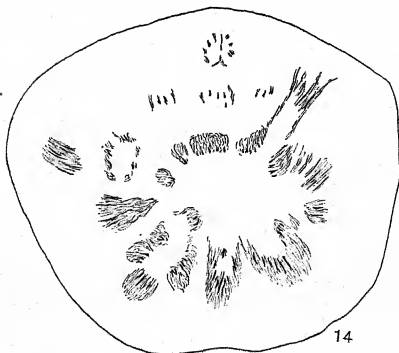
Figs. 12-29. Caesalpinoideae (continued). All from transverse sections taken at successively higher levels. 12-21. *Cassia laevigata* Willd. 12. Flower base. 13. The same at the level of origin of the cords for the postero-lateral sepals. 14. The same after exertion of the postero-lateral sepals. The vascular cords for the "standard" and "wing" petals and for some of the back stamens have turned out from the central ring, those for the remaining members of the perianth and androecium are in process of differentiation. 15. Anterior portion of the same. In the centre the residual vascular ring serving the gynoecium, and around it the vascular complexes for the seven front members of perianth and androecium in various stages of differentiation. 16. The same. The residual vascular ring shows a strongly developed bundle in the mid-line in front and a weak one in a corresponding position at the back. 17. The same. The posterior median strand has been replaced by a pair of finer strands. 18. The stipe now free. The ring of vascular bundles is surrounded by a distinct endodermis and pericycle. Phloem still persists in the posterior median sector, but xylem elements are no longer traceable in this position. 19. The ovary. Endodermis and pericycle have undergone disintegration. The phloem in the posterior median line has now died out, leaving a wide gap. 20, 21. Vascular tissue from the filament of one of the stamens standing on the radius of a "keel" petal. 20. From the base of the filament. 21. From the apex of the filament. 22-29. *Cassia corymbosa* Lam. 22. The stipe with even, convex, posterior contour. The vascular ring, surrounded by endodermis and pericycle, shows a median bundle with a pair of laterals both back and front. 23. The same. The median, posterior xylem strand has disappeared, with consequent indentation in the vascular ring and flattening of the contour line on this radius. 24, 25. The ovary immediately before, and after, the appearance of the loculus. All vascular elements have now disappeared from the posterior sector and the outer contour is becoming increasingly concave. 26, 27. The same. The ends of the interrupted limiting layers are directed outwards towards the periphery. The outer posterior contour first becomes flattened, and then convex, as the immediate effect of the disappearance of the vascular tissue from the middle line is gradually overcome. 28. The same. The placental strands are beginning to arise and are directed towards the middle line, the outer contour consequently continues to be convex. 29. The ovary apex. The twin postero-lateral cords have come to an end and the ventral furrow has consequently reappeared. [Figs. 12-17 same magnification. Figs. 18, 19 and 22-29 more highly magnified.]



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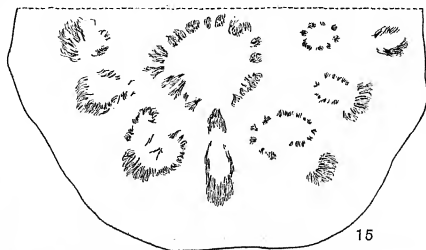


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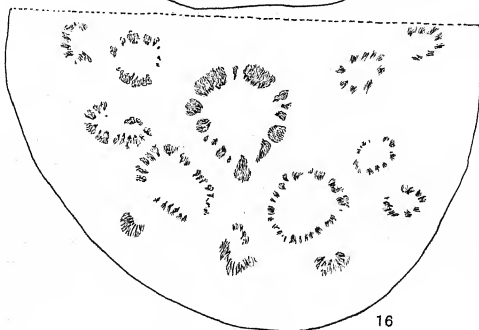


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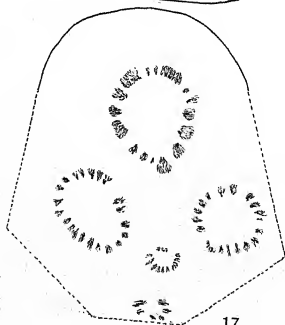
Figs. 12-14



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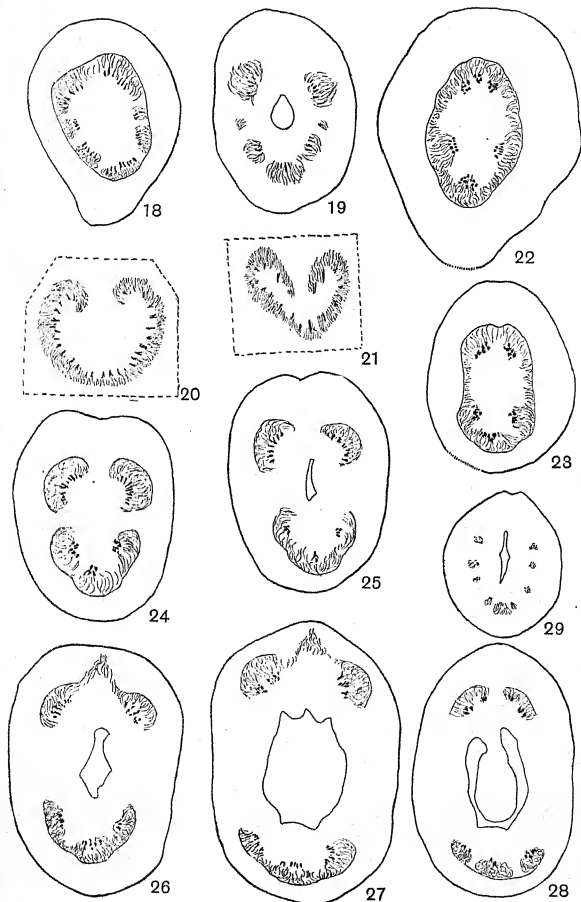


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Figs. 15-17

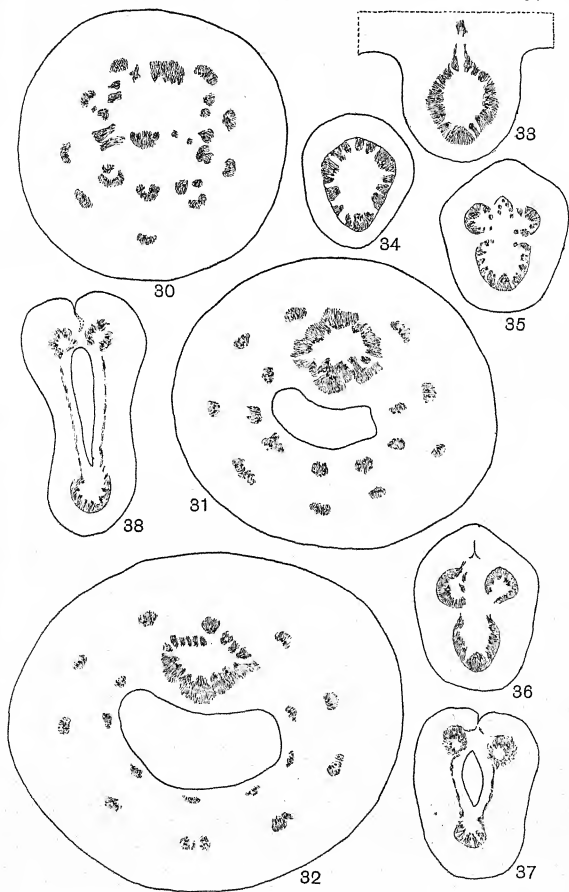


Figs. 18-29

the anterior and posterior halves of the flower is still further emphasised. The ovary is markedly excentric adhering at its base to the posterior wall of the flower tube, with the result that the bundles running to the perianth and stamens leave the central cylinder in succession, in order from front to back. Further, as will appear, the mode of origin of the vascular system of the gynoeceum in *Saraca* (and sometimes apparently in the case of *Brownea*) differs in certain respects from that described in any of the preceding types.

Saraca indica L. (Figs. 30-38). This genus is remarkable in that the perianth is composed of four orthogonal, sub-equal, petaloid structures. Nevertheless the full number of perianth cords (10) is given off from the central cylinder. This is also the case in the androecium although two or three of the hindmost stamens are aborted and merely complete the basal region of the staminal tube. The anomaly of the perianth affords one more instance among countless others where the vascular system provides the clue to the explanation. *The four perianth structures in SARACA are not equivalent to any four members of the normal caesalpinoid perianth.* The anterior-posterior pair receive each three vascular components which in the normal type enter three separate perianth members—the anterior sepal and “keel” petals in the one case, the “standard” petal and postero-lateral sepals in the other. The lateral pair receive each the cords which normally supply one antero-lateral sepal and one “wing”

Figs. 30-38. Caesalpinoideae (*continued*). All from transverse sections taken at successively higher levels. *Saraca indica* L. (from a specimen with seven stamens). 30. Flower base. At the front and sides the cords for the seven front members of the perianth and some of the androecium; those for the remaining members are not yet differentiated. In the centre a belt of vascular strands stretches across the pith from side to side. 31. The same after the appearance of the cavity of the flower tube. The transverse belt of vascular strands has become incorporated in the reformed vascular ring. 32. The same at the level at which the cord for the posterior perianth member (“standard” petal) leaves the reformed ring. 33. A portion of the flower wall with the projecting ovary stipe not yet disjoined. At the back the cord for the posterior petal. 34. The stipe, now free, with an even rounded outline. An endodermis and pericycle surround the vascular ring. 35-38. The ovary. 35. After these layers have undergone disruption on either side of the posterior median sector and also in the lateral plane. The vascular complex on either postero-lateral radius is becoming arranged in a ring. 36. The ends of these disrupted layers bordering the posterior median line are now directed outwards towards the periphery. The vascular complex on the postero-lateral radii shows a more definite ring arrangement. The intervening posterior sector is now destitute of vascular tissue. 37, 38. The disappearance of the vascular tissue from the posterior sector has been followed by the appearance of a ventral furrow. The vascular elements are becoming withdrawn from the side walls of the ovary and consolidated with the anterior and two postero-lateral arcs. [All magnified equally.]



Figs. 30-38

petal. Hence the actinomorphic symmetry of the perianth and the reduced number of its members. This grouping of the vascular cords for the perianth finds a parallel in some of the Papilionatae (see later, p. 244 and Fig. 66). In this latter case, however, the change is not from the separate condition to the associated, but the reverse, the 10 cords arising in the first instance in four groups (3, 3, 2, 2), the components becoming disjoined later so that the full number of perianth parts is present, each member individually taking shape.

The first indication of differentiation of the vascular system of the gynoeceum can be observed almost as soon as the first bundles turn out from the central vascular cylinder for the perianth. At this level a disconnected patch of fibres appears in the pith. True vascular elements presently come to be associated with these fibres. Other strands arise, forming a chain across the pith, dividing it into an anterior and a posterior half (Fig. 30). The cavity of the flower tube presently makes its appearance in the front area of the pith, and the transverse belt of vascular strands is now withdrawn more to the back (Figs. 31, 32). After the last cords for the stamens and perianth have passed outwards, the reformed central ring continues entire up into the short stipe which is eventually disjoined from the flower wall, the ventral face being detached last (Fig. 33), the order of separation being thus the reverse of that in *Cassia* (see Fig. 17). In cross-section the free stipe presents an even contour line and a central cylinder delimited by what appears to be a pericycle of several layers and a bundle sheath, both rapidly becoming crushed (Fig. 34). Some of the bundles composing the vascular ring are larger, some smaller, but there is at first no obvious indication of the later differentiation into two carpel systems. As the stipe assumes a more definitely ovoid outline the vascular ring becomes diamond-shaped and then breaks across on each side in the lateral plane, forming two separate arcs. Xylem elements disappear from the median posterior sector, a few scattered phloem strands alone remaining. Bundle sheath and pericycle rupture between these remnants and the two well-developed postero-lateral bundles (Fig. 35), and the separate, median, crushed portion becomes pushed to the periphery (Fig. 36) as in *Cassia*. Owing to the distance from the surface at which these two bundles are situated the effect of these changes is not immediately reflected in the contour line, but at the level of the loculus the sinking-in on the posterior median radius is well marked (Fig. 37). In the meantime the elements lying nearest to the middle line in each of these two bundles turn inwards (Fig. 35) and by a process of invagination,

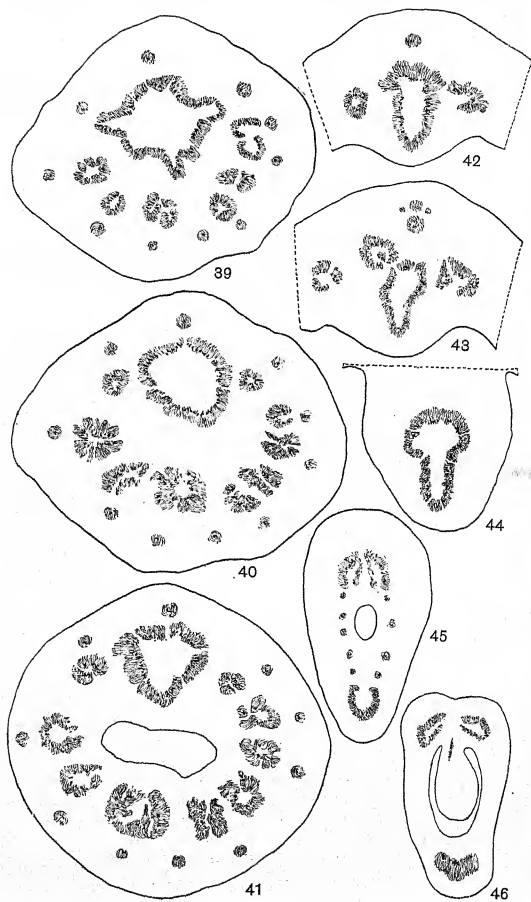
as it were, each complex comes to be formed into a more or less complete ring—a configuration difficult to reconcile with the orthodox conception that these stele-like cords are but the marginal veins of a folded leaf. At the same time the vascular elements in the side walls draw apart to the back and front so that the two vascular systems centred at the poles become distinct (Figs. 37, 38). Rupture of the ovary wall in the posterior median line readily occurs by tearing of the parenchyma cells lying between the disconnected ends of the pericycle and separating the ventral furrow from the loculus.

Brownea coccinea Jacq. (Figs. 39–46), *B. Crawfordii* W. Wats. (Figs. 47–53). In this genus the stamens vary in number from 10–15. In the flower of *B. coccinea* from which the accompanying drawings were taken the number was 12; 13 were found in other specimens, and 11 in several flowers of *B. Crawfordii*.

As in *Saraca* the ovary is adherent to the posterior wall of the flower tube. In *B. coccinea* the cords for the back stamens emerge from the central cylinder considerably later than those for the stamens nearer the front (Figs. 39–43). After their departure the residual cylinder appears in the form of an ellipse with the long axis in the median plane¹ (Fig. 44). A notch in the outline on each side foreshadows the break at these points which occurs shortly after the ovary stipe has become wholly free, the ventral face, as in *Saraca* (Fig. 33), being the last to be detached (Fig. 44). As the ovary assumes the characteristic ovoid shape in cross-section the bulk of the two sectors of vascular tissue, including the whole of the xylem, converges to the two poles, only a few scattered phloem elements remaining in the side walls (Fig. 45). At the ventral pole the xylem elements shortly disappear from the middle line and two postero-lateral rings are formed by the same process of invagination as in *Saraca*, thereby leaving the middle line devoid of vascular tissue. As these two ring complexes come to lie further apart the ventral contour, hitherto convex, becomes concave (Fig. 46).

B. Crawfordii resembles *B. coccinea* in all essential features and a detailed description of this species is unnecessary. Stages supplementing those shown for *B. coccinea* are represented in Figs. 47–53.

¹ Apparently the stages preceding the appearance of the ellipse vary somewhat in different specimens, since McLean Thompson figures a case ("Studies in advancing Sterility, I," p. 11, Fig. 12 IV, in *Publications of the Hartley Botanical Laboratories*, 1, University of Liverpool) in which a bar of vascular tissue is shown extending across the middle of the central parenchyma in the manner of the chain of strands described in the present account as observed in *Saraca* (see p. 238 and Fig. 30).



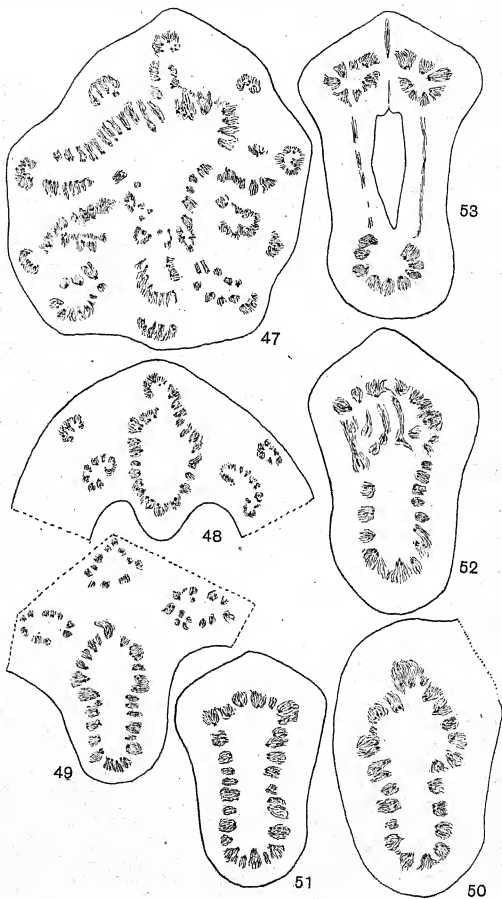
Figs. 39-46

Schotia speciosa Jacq. This species shows much the same appearances in the development of the gynoecium as *Brownea* and calls for no special comment.

Poinciana Gillesii Hook. (Figs. 54-56). In this type the gynoecium is nearly central. At the level at which the stipe is all but disjoined from the flower wall the residual vascular tissue forms a completely continuous ring in which xylem elements occur at intervals all round the circle, and are so distributed that their disposition gives no certain indication as to which sector will prove to be the anterior one and which the posterior (Fig. 54). At a higher level it becomes evident that the ventral face is the last to be detached, and this fact established, the anterior-posterior orientation in the earlier stage can then be deduced. As the free stipe becomes ovoid in cross-section the xylem elements are seen to be collected into an anterior and a posterior sector (Fig. 55). In the former they lie at first on an extended arc, becoming concentrated later into a central bundle and a pair of laterals. In the latter they consolidate into twin cords. Increase in the anterior-posterior diameter is followed by the usual changes. The continuous phloem belt becomes interrupted. As the portion in the mid-line at the back dies out the ventral surface becomes concave. The long band on each side connecting the two polar systems breaks up into numerous small bundles (Fig. 56).

Haematoxylon campechianum L. Only young buds of this species were available for investigation, but, so far as appeared, this type resembles *Poinciana* in respect to the points dealt with above.

Figs. 39-46. Caesalpinioideae (*continued*). All from transverse sections taken at successively higher levels. *Brownea coccinea* Jacq., from a flower having 12 stamens. 39-41. Flower base. 39. The vascular cords are differentiated for all the ten perianth members and for the seven stamens nearest the front. 40. Vascular cords for two more stamens (8th and 9th) have been given off from the residual ring. 41. The cavity of the flower tube is seen in front of the residual vascular ring, exposing the anterior surface of the gynoecium. 42, 43. Posterior portion of the flower base. 42. The cord for the 10th stamen has just been given off to the right. At the back the cord for the "standard" petal. 43. The cords for the 11th (left) and 12th stamens are now seen in addition to the two appearing in 42. 44. The free portion of the ovary stipe showing a complete vascular ring. 45, 46. The ovary. 45. The vascular ring is in process of breaking up; the xylem elements are becoming aggregated at the two poles; the vascular complex on either side of the middle line at the back has begun to take on a ring form. These ring formations lie near to the middle line, and the posterior contour line is consequently even and convex. 46. These ring formations now lie further apart, and all vascular elements having disappeared from the intervening sector the ventral outline shows a median furrow.



Figs. 47-53

In the disposition of the xylem elements in the ventral vascular sector of the ovary stipe in certain Caesalpinoideae, as in the Mimosoideae, we get a most valuable clue to the origin of that most characteristic feature of consolidated fertile carpels—the arrangement of the vascular elements in twin cords instead of in a single main bundle. For the appearances above described, especially those in *Acacia*, *Albizzia* and *Cassia* indicate that the venation system may develop, up to a point, on the same lines in a consolidated carpel as in the typical valve carpel, giving rise to a central bundle and a pair of laterals in both cases. But whereas, in the typical valve carpel which, when present with solid carpels, is generally sterile, the form of expansion is such as to lead to the predominance of the median over the lateral bundles, in the consolidated type which, when present with valve carpels, is commonly fertile, exercise of the reproductive function has led to degeneration and ultimate disappearance of the centre bundle and to the emphasised development of the fertile laterals.

Further, the evidence clearly points to an intimate relation between the contour of the ovary and the distribution of the vascular tissue, and more particularly of the xylem elements. The effects of this distribution manifest themselves after the gynoeceum has become free from the flower wall, and after rupture of the endodermal and pericyclic layers (where these are present) allows full play to the forces bringing about extension. The rupture of these layers and the almost simultaneous occurrence of the tissue split that heralds formation of the loculus must inevitably involve a considerable change in the tissue tensions. After the ovary (or stipe) has become free the greatest extension takes place in the direction of the lines joining the two postero-lateral cords with the anterior cord. The effect of this increase in diameter along the arms of a V is to cause the convex outline of

Figs. 47-53. Caesalpinoideae (*continued*). All from transverse sections taken at successively higher levels. *Brownea Crawfordii* W. Wats. 47. Flower base at the level of differentiation of the perianth and the front stamen cords. 48, 49. Posterior portion of the flower base after the cavity of the flower tube has appeared. 48. Before differentiation of the vascular cord of the posterior stamen. 49. After differentiation of the same. 50. The ovary stipe immediately after it has become free from the flower tube, showing an even rounded outline except where it has recently become disjoined (indicated by dotted line). 51. The same. The contour reflects the change in shape of the vascular ring. 52, 53. The ovary at a stage just before, and just after that shown for *B. coccinea* in 45. In 53 the (? pericyclic) layers bounding the two postero-lateral ring complexes have become disrupted in the middle line, and the broken ends are directed outwards towards the periphery (seen as a thick, median streak).

the sector lying between the open arms of the V to become concave, hence the ventral furrow. The appearance of the loculus, one cannot but suppose, must also have an effect, over and above that due to the absence of vascular elements in the middle line, reducing the capacity of the remaining parenchyma cells on this radius to overtake the increase in diameter which has by now taken place on the radii of the two postero-lateral bundles. In various papilionaceous types (see later, p. 245) the difference in tension and rate of extension on these radii at this level may be such as to cause a shallow ventral furrow to become a channel so deep that it and the loculus become continuous. But just as in those types which develop only a shallow furrow adjustment of these differences takes place so rapidly that the furrow is almost immediately obliterated, so, in those extreme cases in which the loculus, immediately that it is in being, comes into communication with the exterior, this condition is only seen, as a rule, in one or two transverse sections. Almost at once the bounding cell layers meet together, the connecting passage is obliterated, and the loculus henceforth is closed.

PAPILIONATAE

Flower zygomorphic, gynoecium central.

In this section, as in the Caesalpinioideae, the order of separation of the ovary surfaces from the flower tube varies in different genera. Thus the anterior face was found to become disjoined last in *Cytisus*, *Hedysarum*, *Oxytropis*, *Astragalus*, the posterior in *Lathyrus*, *Coronilla*, *Medicago*, *Ononis*.

The manner of emergence of the perianth and stamen cords points to a general process of speeding-up. For example, two or three perianth cords together with those for the superposed stamens in some types leave the central vascular cylinder as a single bundle mass. Thus in *Oxytropis argentata* Pers. (Fig. 66), *Hedysarum multijugum* Maxim., *Lathyrus setifolius* L. the whole twenty bundles serving perianth and androecium issue as four such masses which turn out in the orthogonal planes, leaving the residual vascular tissue in the form of four diagonal quadrants (Fig. 66). It will be obvious, in view of the fact that this kind of condensation varies in degree, that variations in the vascular arrangement following *immediately* upon the emergence of the bundles for the outer whorls, and before reconstitution has taken place, are not invariably significant.

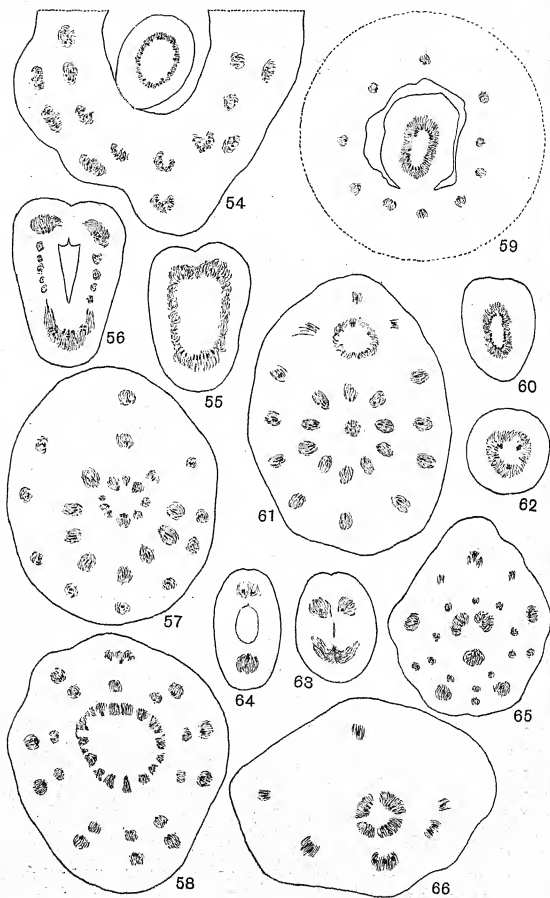
As the residual bundle masses serving the gynoecium become rearranged they appear seldom, if ever, to constitute a *continuous* ring

such as has been described above as occurring in certain Caesalpinioideae. This may be due to the fact that in nearly all the Papilionatae which were examined the stipe region is but little developed. Where the residual vascular tissue is fairly considerable in amount it usually consists of numerous *separate* bundles arranged in a ring or in a horseshoe pattern with the opening turned towards the ventral face. In some genera showing the former arrangement a bundle is situated in the middle line at the back which still develops a xylem component, a significant feature in the present connection, as has already been made clear in the above account of the Mimosoideae and Caesalpinioideae. This condition was observed in *Colutea arborescens* L. (Fig. 57) but is apparently rare. On the other hand, phloem elements without accompanying xylem occur in this position in many types (see Fig. 58). In other cases the whole of the vascular tissue situated in the posterior median line passes out in the trunk cord destined for the "standard" petal and superposed stamen, leaving a permanent and wide gap (horseshoe pattern) as occurs in *Oxytropis argentata* Pers., *Ononis fruticosa* L. and *Lupinus* spp.

But whatever the initial differences in the distribution or arrangement of the vascular tissue in these several types, further development proceeds on the same lines. As in the Mimosoideae and Caesalpinioideae the vascular elements become concentrated into an anterior arc which, usually, is shortly resolved into a centre bundle and a pair of laterals, and a posterior arc composed of twin bundles, one on either side of the mid-line. In genera where the residual tissue is of minimum amount this stage may be reached at once after the exit of perianth and stamen cords as is the case, e.g., in *Coronilla elegans* Panč. (Fig. 65), *Medicago marina* L. (Fig. 67), *Astragalus hamosus* L.

Cytisus filipes Webb and Benth.

As mentioned above, tissue distribution and tissue tensions at the gynoeceum base are sometimes (? generally) such that growth and extension lead to actual communication by means of a narrow channel between the ventral surface and the loculus at the moment that the cavity makes its appearance in the central parenchyma. This condition would seem, however, to be rather accidental than significant, depending largely upon the number of cell layers that intervene between the surface and the spot where the tearing apart of the parenchyma cells first begins. In *Cytisus filipes*, where there is no stipe development, it may come about at the moment that the ovary becomes free along its ventral face. In *Crotalaria capensis* Jacq.



Figs. 54-66

a short stipe is present. This shows at first, in cross-section, an even circular outline and an unbroken endodermal and pericyclic ring surrounding an anterior and two postero-lateral bundles. The rupture of this ring is at once followed by considerable extension in the antero-posterior diameter through a rapid increase in the amount of parenchymatous tissue. The distance between the anterior and the postero-lateral cords is almost immediately doubled. On the posterior median radius itself the effect of this extension is to cause an invagination¹ in the form of a narrow channel of considerable depth. At the same time, at a point only some three or four layers internal

¹ Although 'invagination' describes the appearance more conveniently than any other term it must be understood, as has already been stated, that it is not used here as equivalent to an infolding, but as indicating the effect produced by a lagging behind in growth on one radius as compared with that on neighbouring radii.

Figs. 54-66. All from transverse sections taken at successively higher levels. 54-56. *Caesalpinioideae* (continued). *Poinciana Gillesii* Hook. (from a specimen with eleven stamens). 54. Anterior portion of the flower wall with the all but disjoined ovary stipe, which shows an even rounded contour. The vascular tissue of the stipe forms a continuous ring with xylem elements distributed at intervals round the circle. 55. The stipe. The xylem elements are concentrated in the front sector and on the two postero-lateral radii. The disappearance of xylem elements from the posterior median line has been followed by the appearance of a ventral furrow. 56. The ovary after formation of the loculus. The vascular ring has broken up, the phloem elements in the side walls forming a chain of separate strands. 57-66. *Papilionatae*. 57. *Colutea arborescens* L. Flower base. The residual vascular ring for the gynoeceum shows a median bundle with xylem back and front, the former small, the latter well developed. 58. *Lathyrus cicera* L. Flower base. The residual vascular ring for the gynoeceum consists of numerous bundles, those nearest the middle line at the back forming no xylem. 59, 60. *Cytisus Adami* Poit. 59. The inner portion of the flower wall and the nearly disjoined gynoeceum. The xylem elements of the vascular ring of the gynoeceum are concentrated in the anterior and posterior sectors. 60. The gynoeceum immediately after it has become free. The absence of xylem elements in the posterior median line is already reflected in the slightly concave outline of the ventral face. 61-64. *Hedysarum obscurum* L. 61. Flower base. The whole vascular arrangement is extremely regular except for the large supply of vascular elements to the "standard" petal, an inequality which gives rise to a zygomorphic ground plan. 62. The ovary stipe with circular contour line. 63. The ovary base at the level of origin of the loculus. The twin posterior cords lie some distance apart, the intervening sector being without vascular tissue, hence the appearance of the ventral furrow. 64. The ovary with fully developed loculus. The twin posterior cords have moved nearer together, being almost in contact, a disposition which brings about the obliteration of the ventral furrow. 65. *Coronilla elegans* Panč. Flower base. The residual vascular tissue for the gynoeceum is reduced from the first to the anterior and the twin posterior cords. 66. *Oxytropis argentata* Pers. Flower base. The vascular cords for the ten perianth members have left the central cylinder as four bundle masses which are breaking up into their component bundles. In the centre four residual bundle masses which serve the gynoeceum.

to the termination of the channel, the parenchyma cells begin to separate from one another (Fig. 68). A small cavity appears, enlarges, and through the consequent interruption of these few intervening cell-layers comes to be continuous with the channel. But this process of invagination is now rapidly counteracted and the channel obliterated, a shallow depression (the ventral furrow) alone remaining. In a species of *Genista* where a longer stipe is developed this structure is at first elliptical in cross-section. The vascular tissue which is bounded by an endodermal and pericyclic sheath shows xylem elements at scattered points round the ring except on the posterior radius where phloem alone is present. In due course the xylem elements become concentrated at the poles, the phloem on the posterior radius disappears, the enclosing sheath becomes ruptured, and the ensuing increase in size from back to front results in an invagination which becomes continuous with the loculus at the moment of its origin, and is then immediately obliterated, leaving merely a shallow furrow.

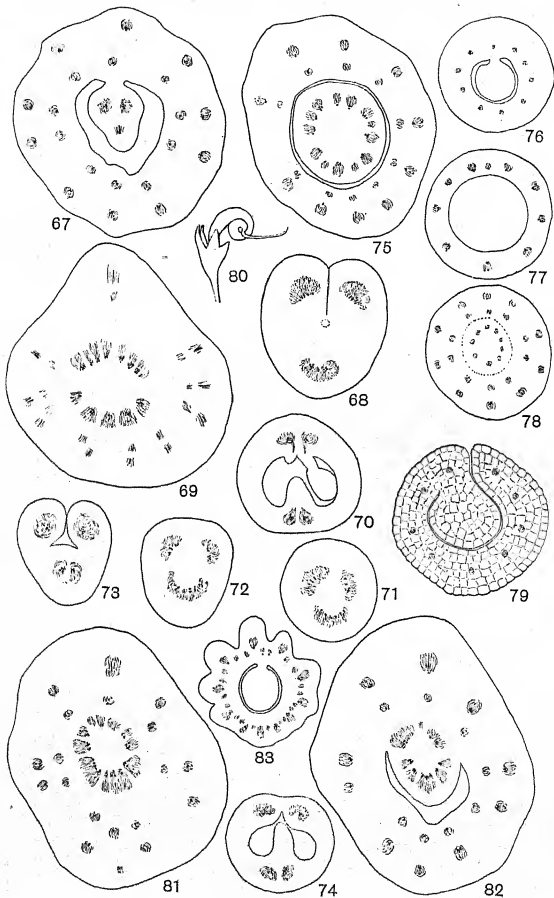
Another type in which this invagination is particularly well seen is one which also calls for fuller consideration from another point of view, viz. *Astragalus*. From the present standpoint this genus is one of the most interesting among the Papilionatae, for here we meet with a phase of the condensation process only observed, so far, in one other case. For not only has the centre bundle of the fertile carpel cord completely disappeared in the species examined but that of the sterile carpel cord is evidently following the same course. It is, indeed, only in a species here and there that we obtain a last glimpse of this latter bundle before it also vanishes, when the equivalence of the two carpel systems becomes most striking (Figs. 73-75). Suppression of both centre bundles occurs also in *Biserrula Pelecinus*.

In *A. monspessulanus* L. (Figs. 69, 70) and *A. sesameus* L. the residual vascular tissue appears in cross-section in the form of an interrupted ring, a break occurring on each side in the lateral plane (Fig. 69). Several xylem strands are present in each arc. The two sectors, indeed, at this level are often so similar that it is scarcely possible by mere inspection to tell which represents the posterior arc and which the anterior. This is the case also in *Cytisus Adami* Poit. (Figs. 59, 60) and, as described above (p. 241), in *Poinciana Gillesii* (Fig. 54) amongst the Caesalpinoideae. In *A. monspessulanus*, at about the level at which the ovary is becoming disjoined from the flower wall, the xylem elements in the anterior sector have become concentrated into a central and two lateral strands. The centre strand divides in half radially almost at once, each half merges with the

lateral strand on the corresponding side and thus two anterior bundles arise which are the counterparts of the twin posterior bundles (Fig. 70). Like the latter the anterior pair continue distinct in their upward course, though usually standing in such close proximity that the anterior, outer contour remains evenly convex. In this species we catch sight for a moment of the mid-line bundle of the front carpel before, by a process of division and coalescence with the near-by laterals, it comes to an end. In *A. sesameus* and *A. frigidus* A. Gray (Figs. 71-73) the front central bundle can no longer be traced. Before the ovary is yet entirely free the two sectors each show only twin bundles. In *A. hamosus* L. the two adjacent bundle masses bordering the gap left by the exit of the single trunk cord supplying the anterior sepal, "keel" petals and the superposed stamens converge, their xylem elements adjoin, with the result that the vascular component of the anterior sector no longer appears as a double structure but continues as a single cord. This condition obtains also for a short distance in *A. frigidus* (Figs. 71, 72).

From these examples of bicarpellary types we may now turn to consider the two exceptional genera *Arachis* and *Scorpiurus* in which also the ovary is truly terminal. It was suggested in the earlier account that some ten carpels have gone to form the ovary in both these genera.

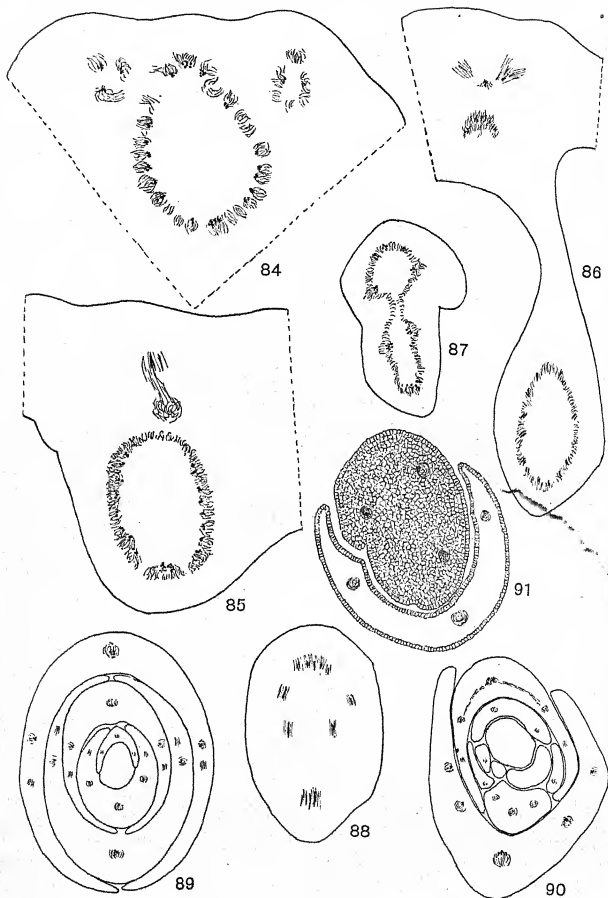
Arachis hypogaea L. (Figs. 75-79). In this species it may be noted in passing that though the full complement of stamens is occasionally present, the posterior median member is frequently lacking. Although, in these circumstances, a vascular strand usually passes out conjoined at first with the vexillum bundle, becoming separate later, no further development of this strand takes place after it has become disjoined, and the stamen it should serve is not formed. The ovary is central and becomes free from the flower wall at all points simultaneously. After the emergence of the perianth cords, which carry out the stamen bundles with them, the residual vascular tissue consists of a ring of numerous separate strands of which ten or more (though sometimes fewer) develop xylem (Fig. 75). These equivalent xylem-forming bundles all persist, taking up a position at about equal distances from one another (Fig. 76) and retaining their individuality throughout the length of the ovary (Fig. 77). When 10 or 12 (the numbers most usually found) of these cords are present, one is situated in the mid-line back and front, the former alone being fertile. The points at which the ovules take their rise become recognisable externally during fruit development through the giving



Figs. 67-83

off of strong laterals from the adjacent cord on either side which converge at this spot forming a nodal complex. It must be emphasised that there is no approximation of any two of the original cords in the manner that we should expect if any pair of them corresponded to the marginal bundles of a single folded carpel. The presence in these circumstances of an *even* number of equivalent cords is also a difficulty on the 1-carpel interpretation, for since the whole configuration has a symmetrical appearance suggestive of an equal development of the two sides a monocarpellary ovary should, presumably, show an *odd* number of primary veins. The fruit is indehiscent in the strict sense, but an irregular ragged splitting of the

Figs. 67-83. Papilionatae (*continued*). All from transverse sections taken at successively higher levels except 80. 67. *Medicago marina* L. The flower wall and the almost free gynoeceium, of which the ventral surface is the last to become disjoined. The residual vascular tissue for the gynoeceium is reduced from the outset to an anterior and twin posterior cords. 68. *Crotalaria capensis* Jacq. The ovary base. The relation of the growth rate to the tissue distribution and the large amount of parenchyma present lead to the formation of a deep ventral channel. The locusus first arises as a small space separated from this channel by only two or three cell layers. 69, 70. *Astragalus monspessulanus* L. 69. Flower base. The residual vascular tissue for the gynoeceium is in the form of two extended arcs, one anterior, one posterior. 70. The ovary. The vascular tissue has consolidated into two pairs of twin bundles, one anterior, one posterior. 71-73. *A. frigidus* A. Gray. 71. The stipe with even circular outline. 72. The same, after the twin postero-lateral cords have diverged considerably. The ventral contour has become flattened. 73. The ovary base. The formation of the locusus in close proximity to the periphery (owing to the large amount of the central parenchyma apportioned to the anterior carpel) causes it to become continuous momentarily with the ventral furrow. 74. *A. Tragacantha* L. The ovary. The ventral furrow is replaced by a shallow depression, as the giving off of the placental strands in the direction of the middle line lessens the width of the non-vascular sector. (For simplicity a small strand in the ovary wall on each side near the lateral plane has been omitted here and in 70.) 75-79. *Arachis hypogaea* L. 75. The flower wall and ovary immediately after disjunction. The residual vascular tissue for the gynoeceium consists of a ring of bundles, ten of which develop xylem. 76. The ovary at the level of attachment of one of the ovules. 77. An unripe fruit. 78, 79. From a very young bud. 78. The flower base. The gynoeceium though delimited is not yet disjoined. 79. The ovary base (highly magnified). The vascular tissue consists of nine equidistant, undifferentiated bundles. There is no bundle in the back median line. The enlargement of the locusus, which makes its first appearance as a small cavity some three or four layers below the surface brings it momentarily into continuity with the ventral furrow. One of the two cords standing nearest to the middle line is already fertile. 80-83. *Scorpiurus sulcata* L. 80. Young fruit. 81. Flower base. The residual vascular tissue for the gynoeceium consists of a ring of bundles, the strands in the posterior sector being without xylem elements. 82. The same at the level at which the ovary stipe is partly disjoined from the flower wall. The phloem elements have nearly disappeared from the posterior middle line. 83. Young fruit. The residual vascular bundles persist as so many equivalent and equidistant cords which severally give rise to a system of lateral veins. At the back the twin placental strands derive from the main cord nearest to the middle line to right and left, respectively.



Figs. 84-91

wall may take place. The tear then occurs alongside the front and back bundles in the median plane, not, as generally in the bicarpellary types, actually in the mid-line by halving the median cords. It appears to be occasionally the case that the residual strand in the middle line at the back does not attain further development but disappears, so that the ovary comes to have an odd number of cords. Examination at a very early stage of an ovary in which this appeared to be the case showed a line of cleavage on the radius of the absent cord leading from the loculus to the exterior (Figs. 78, 79), but like the ventral furrow in bicarpellary types this cleft shortly became obliterated.

Scorpiurus (Figs. 80-83). In this genus the ovary becomes free first along the dorsal and last along the ventral face. At the level at which it is becoming disjoined the residual vascular tissue consists of a ring of bundles showing numerous strands with xylem and phloem at points round the circle, except at the back, where phloem only is formed (Fig. 81). These all-phloem strands shortly come to an end (Fig. 82). The xylem-forming strands, usually 9 but sometimes some higher, odd number, persist, retaining their individuality and extending to the top of the ovary. One bundle is situated in the mid-line in front facing a gap in the centre line at the back, the rest being distributed regularly along the two sides. At a level slightly above that at which the ovary becomes disjoined a strand is detached from each of the cords on either side of the mid-line at the back (Fig. 83). These strands supply the ovules. For reasons similar to those advanced in the case of *Arachis* it seems most satisfactory to interpret the above-mentioned nine, or more, persistent cords as the midrib bundles of a corresponding number of carpels of which the anterior member may develop an extended system of lateral veins (*S. vermiculata*) and is therefore to be classed as a valve carpel, the

Figs. 84-91. Papilionatae (*continued*). All from transverse sections. 84-87. *Baptisia alba* R. Br. 84. Posterior portion of the flower base showing the cords for the two postero-lateral sepals and that for the "standard" petal about to leave the residual vascular ring. 85. The same after this latter bundle has moved out from the ring. In front the anterior face of the ovary stipe, now partly free from the flower wall. 86. The ovary stipe as it bends away from the flower wall cut obliquely. 87. The free stipe becoming transversely constricted preparatory to the formation of two stipes by a process of "twinning." 88-91. From the vegetative stem. 88. *Cytisus* sp. The stem apex. The whole of the vascular tissue is differentiated into a mid-vein and pair of laterals for each of the last two leaves, which have not yet taken shape. 89. *Trifolium* sp. Terminal bud. 90, 91. *Coronilla* sp. 90. Terminal bud. 91. Tip of a bud more highly magnified, showing the midrib and laterals for the last two leaves.

rest being nearer the consolidated type. In the earlier account¹ the view was advanced that the later-formed twin strands in the back middle line represented the double bundle of the cord of a posterior median carpel which otherwise must be supposed to be missing. It seems now, however, in view of the fact that other vascular strands are to be found in this region at a lower level which fail to develop xylem and die out, that these latter strands are the elements of the vanishing median posterior median carpel which is not developed, and that the twin strands which arise later represent, not a whole carpel cord, but two placental strands derived from each of the two back carpels on either side of the middle line.

DISCUSSION AND SUMMARY

The solitary ovary of the Leguminosae is terminal in the strict sense, resembling in this respect the single drupe of *Prunus*. Leguminous types differ, however, from the rosaceous genus in that in them none of the residual vascular elements find their way into the pith and are discarded. These facts established, it becomes necessary to consider their bearing on the problem presented by the gynoeceum.

1. The orthodox view that the legume is formed of a single carpel requires us to accept that the axis is terminated by a well-developed leaf member, an admitted anomaly.

2. This view further requires us to suppose that this carpellary leaf has often, if not invariably, a circular exsertion, a condition not exhibited elsewhere in the plant (see Figs. 88-91).

3. Again, this view, which regards all ovaries of leguminous plants as being alike in constitution, takes no account of the different positions from which the vascular elements for the gynoeceum are derived, or of the fate of these elements during the process of fashioning the ovary.

4. It leaves unexplained the fact that in some genera the outer contour of the ovary is bisymmetrical, showing a similar furrow back and front; also, that the vascular system of the front half is in some cases the counterpart of that in the posterior half.

5. It offers no explanation of the presence in many leguminous genera of vascular elements (seen also in *Prunus*) which persist for a longer or shorter time in the posterior sector between the twin bundle masses which continue upwards as the twin components of the ventral cord, a fact difficult to reconcile with an interpretation which must hold these components to be marginal. Further, it disregards

¹ *Ann. Bot.* 39, 442. 1925.

the obvious difficulty involved in conceiving these twin components, in cases where they show a stele-like arrangement, to be comparable with the individual marginal veins of a foliage leaf.

6. It takes no account of the inability to develop xylem characteristic in many genera of the residual vascular strands lying in the lateral sectors, or of the merging of these strands, except in *Arachis* and *Scorpiurus*, with the dorsal and the ventral cords.

7. It provides no explanation of the ground plan underlying the reorganisation of the residual vascular elements of the ovary into a centre strand and a pair of laterals in the mid-line *both back and front*.

On the other hand, these and many other facts fall into line and present no difficulty on the interpretation of the evidence given below, from which it will appear that the legume is never a monocarpellary structure.

8. The residual vascular tissue available for the gynoeceium, consisting, in general, of a ring of bundles, sometimes surrounded by pericycle and endodermis, represents elements which in some ancestral form were utilised to furnish the midrib cords of two pentamerous carpel whorls.

9. In *Arachis* this ancestral condition is still traceable, in so far that the residual vascular elements come to form (in the typical case) ten strands, which take up a position in line with the ten stamen and ten perianth cords. In some individuals as many as twelve cords may be formed, or, on the other hand, the posterior median bundle may die out (like that of the posterior median stamen) leaving only nine. These cords run separately to the gynoeceium and retain their individuality throughout the length of the ovary. Where all the cords persist, one is situated in the middle line both back and front. These facts point to the conclusion that in *Arachis* 10 (or 12 or 9) equivalent cords which the gynoeceium receives represent so many carpel midribs, a conclusion conveniently expressed by the formula $G = \pm 10$.

10. A similar construction is to be found in *Scorpiurus*, where, however, the posterior median cord probably always fails to develop, hence the gynoeceium receives an odd number of carpel cords ($G = \pm 9$), one standing in the median plane in front, and the one on each side the middle line at the back giving rise to a placental strand. The carpels are either contracted valves or solid, except when the anterior median carpel expands laterally, assuming the proper valve form as in *S. vermiculata*.

11. In other leguminous genera the residual vascular strands on the several radii do not retain their individuality. As a rule, those in the lateral sectors fail to develop xylem and, sweeping towards the median line, become merged in the anterior and posterior cords. Those in the anterior sector become concentrated about the middle line and in the typical case develop into a central strand and a pair of laterals. In the exceptional types *Astragalus* and *Biserrula* the centre bundle is lacking, hence the anterior cord comes to consist of twin bundles, thus resembling the posterior cord, in which the median strand almost invariably disappears in all types at a very early stage. Thus the ovary in all cases except *Arachis* and *Scorpiurus* possesses a dual vascular system, the two systems being based on an identical ground plan, viz. that of a centre strand with a pair of laterals. These relations indicate that in such types $G = 2$.

12. In bicarpellary ovaries the fertile member is semi-solid. It may furnish almost the whole of the ovary wall, in which case the sterile member is a mere column (*Medicago*), or it may contribute only about half the circumference, the sterile member then taking on an extended form and becoming also semi-solid (*Haematoxylon*). Both types contrast with *Prunus*, in which the fertile carpel is solid and the sterile carpel a valve.

13. The bicarpellary construction of the gynoeceium in all types except *Arachis* and *Scorpiurus* throws light on the manner of dehiscence of the fruit. Where the two carpel systems are about equally developed and remain unconnected or form but few anastomoses, splitting may take place by irregular tearing of the tissues in the middle of the two flat sides of the pod, i.e. at the limits of the two systems as in *Haematoxylon campechianum*. Where the two carpel systems become interconnected by copious branching, dehiscence is most easily accomplished by the splitting in half of both posterior and anterior midrib cords, through the drying up of the parenchyma which forms a medullary ray between the twin bundles in both cords after the disappearance of the centre strand. If the dorsal cord becomes consolidated by a cap of fibres and forms few or no lateral branches while the ventral cord forms a number of strong secondary veins, dehiscence most easily occurs alongside the dorsal cord, as in *Medicago*. If both cords become consolidated by the development of a strong enveloping arc of sclerenchyma, and but few lateral veins are present as in *Carmichaelia*, the two cords may persist intact as a frame. The few weak lateral veins are easily snapped, and the sides of the pod become detached on drying and fall from the frame.

Where there is a multiple vascular system with numerous anastomoses, as in *Scorpiurus* and *Arachis*, the fruit usually remains indehiscent, though tearing of the tissues *alongside* the median cord both back and front may occur in old, dried pods of *Arachis*.

14. The fact that the gynoeceium in the Leguminosae, as in the Rosaceae, becomes disjoined from the flower wall so that in some genera the ventral, and in others the dorsal face is the last to become free, is more easily comprehensible if it is a question of sometimes the one, and sometimes the other, of two leaves situated on opposite sides of the axis becoming detached last from the flower wall, than if this variation implied that a particular solitary leaf member became free from the surrounding leaf whorls, sometimes by its midrib before its edges, and sometimes *vice versa*.

15. The presence of a second ovary was found to be due, in the only case (*Baptisia*) in which material was obtainable for investigation, to bifurcation of the stipe below the level of the ovary proper, both gynoeceia having a normal structure (see Figs. 84-87).

The conclusion that all leguminous types, when not multicarpellary (*Arachis* and *Scorpiurus*), are bicarpellary, has further implications of great interest from a wider aspect. They are as follows:

16. The appearance of a furrow in the outer contour of the leguminous ovary, hitherto regarded as indicating the line of junction of the edges of a folded carpellary leaf, is not a sign of this construction but arises as the result of an interruption in the endodermal and pericyclic layers, the absence of any xylem elements on the radius of this break, and the consequent slight extension on this radius compared with that on neighbouring radii, on which there is considerable development of xylem tissue. The fact that this ventral furrow may become continuous, momentarily, with the developing loculus arises from three sets of circumstances, (a) the slight rate of extension taking place on the posterior median radius as compared with that on the adjacent postero-lateral radii, (b) the nearness to the periphery at which splitting apart of the parenchyma cells to form the loculus takes place in the first instance, (c) the inability of the few cell layers external to the loculus on the posterior median radius to keep pace at this stage, by growth or division, with the enlarging loculus sufficiently to maintain a continuous boundary.

17. The vascular anatomy of the legume throws fresh light on the characteristic difference between the venation system of the valve and the consolidated carpel, and, incidentally, on the position of the ovules in the two types. Both may be presumed to trace back to

the same ground plan, viz. a centre bundle with a pair of laterals. In a valve carpel, which has often considerable lateral extension and has in many cases become sterile, the centre (= midrib) bundle is usually more highly developed than the laterals (= marginal veins). In the consolidated type, which often has little lateral extension and is very generally fertile, the centre bundle has degenerated and in many cases disappeared altogether, while the twin laterals which supply the ovules reach a large size. If these twin bundles, which run a parallel course close together, are the homologues, wholly or in part, of the marginal veins of the valve carpel, the position of the ovules in the consolidated carpel becomes intelligible.

18. Study of the leguminous ovary bears out the conclusion formed after investigation of the ovary in Berberidaceae¹, in certain Rosaceae², in Typhaceae and Sparganiaceae³, in Pandanaceae⁴, in certain Phytolaccaceae⁴, and in the Gramineae⁵, in fact, in all types so far examined which have been held to possess an ovary formed from a single terminal carpel, viz. that in no case is this anomaly a reality. In other words, the gynoeceum is not in any of these cases morphologically equivalent to a single leaf in the same sense in which all other types of leaf, whether floral or vegetative, are equivalent to one another. In *Arachis* and *Scorpiurus* the ancestral, pentamerous ground plan of the gynoeceum is still traceable. In the remaining genera of the Leguminosae, though the elements which once (no doubt) developed into the vascular system of ten carpels still survive in whole or in part, and are utilised, they are now fashioned into the venation systems of a median pair of carpels only. In this connection I am tempted to employ a simile which I have used before, but I can think of none more apt. In the breed of swine described as "mule-footed," the skeleton shows the bones of two toes although the hoof is undivided. So in the typical leguminous ovary the vascular system shows a dimerous ground plan, although the outward appearance simulates in some respects that of a single folded leaf.

The accompanying drawings were made by Miss D. F. M. Pertz, to whom I here tender my very grateful thanks. I am much indebted also to Mr W. J. Dowson for specimens from the R. H. S. Gardens at Wisley of 2-podded flowers of *Baptisia*.

¹ See "Illustrations of Carpel Polymorphism, II," *New Phyt.*, 27, 175. 1928.

² See "Carpel Polymorphism, II," *Ann. Bot.*, 41, 569. 1927.

³ Account in the press.

⁴ Account not yet published.

⁵ See "Carpel Polymorphism, I," *Ann. Bot.*, 39, 155. 1925; and "Illustrations of Carpel Polymorphism, I," *New Phyt.*, 27, 59, Fig. 55. 1928.

REVIEW

Plant Diseases, by F. T. BROOKS. Oxford University Press: London, Humphrey Milford, 1928. Pp. vi + 386, with 62 Figures in the Text. Price 21s.

English students of plant pathology, and especially those whose work lies in the British Isles, will welcome the appearance of this book—an appearance which, judged by the frequency of textbook publication in other countries, is long overdue. With the rapid growth of the subject, which has been especially pronounced since the War, the textbooks quickly become obsolete. New diseases are described; the relative importance of others changes as time goes on; the old established diseases are more fully worked out. Even within a decade or so, entirely new conceptions have arisen, e.g. that of the filterable virus as related to plant disease. It is almost incredible that the virus diseases, which figure so prominently in the present-day literature of plant pathology, are not even mentioned in Massee's *Diseases of Cultivated Plants and Trees* (1910 edition), the standard textbook of twenty years ago for English students.

During the somewhat long interval from Massee to Brooks, the teacher of plant pathology has had no choice other than to recommend to his students textbooks of foreign origin. Whatever merits the latter may have had—and in some cases they are considerable—there is nevertheless the great disadvantage that the foreign textbook deals largely with conditions that are more or less different from those obtaining in England. Thus diseases of great importance in one country are unknown or unimportant in another. Contrast for example the relative importance of pear "blight" and of "wart" disease of potato in England and in America respectively. Therefore while the principles of plant pathology are much the same everywhere, the details of treatment and the amount of emphasis to be placed on particular diseases must be different in the textbooks of different countries, if a proper balance is to be preserved and the particular requirements of the reader met.

The arrangement of the subject-matter is on the basis of the causal agent of the disease. For the purposes of a general textbook, this is still the best arrangement. After a brief introduction dealing with general matters such as disease symptoms, nature of resistance, mode of dissemination of disease, etc., there follow chapters on non-parasitic diseases, virus diseases, diseases caused by Bacteria, by Actinomycetes and by Myxomycetes. The greater part of the book deals with diseases of fungal origin, arranged according to the more recent classifications of the Fungi. The final chapter gives a short account of fungicides. There is a good general index.

The diseases listed and described in more or less detail include all those of any consequence which are known to occur on cultivated plants in Britain. A selection is also included of some of the more important or more interesting foreign diseases. Confining attention to those of fungal origin, we may remark that over four hundred are described, and that within the limits of 290 pages. The description in each case is thus necessarily very brief. Even an important disease like potato blight receives no more than three pages. But while the dominant note is brevity, this has been achieved without any sacrifice of essential information. This book is in fact packed with an amazing amount of up-to-date information. Every sentence contains a fact. There is no reading of the easy armchair sort; there are no literary flourishes and no wordiness.

The descriptions, which are well proportioned to the relative importance of the particular disease, have a further noteworthy and highly commendable feature. This is the inclusion, for each parasitic genus and for most of the species, of a concise diagnosis of the parasitic organism. For most of the diseases occurring in Britain, the common names suggested or preferred by the

Plant Pathology Sub-committee of the British Mycological Society are given. By including these names, the author is doing a good work in bringing nearer the realisation of a uniform common terminology of plant diseases.

Throughout the text there are numerous references to original authorities. These are grouped into extensive bibliographies appended to each chapter, and form most valuable additions. The author has collected diligently, and the student who desires fuller detail on any subject has an easy path before him.

The illustrations, which are all original, are inserted somewhat sparingly; that is, in comparison with other textbooks of the kind. Considerations of space and cost are no doubt all-important, and the author of a textbook of this description must have great trouble in deciding what to include by way of illustration and what to omit. It is clear that adequate illustration of even a moderate proportion of the diseases described is impossible. While some readers would consider the book as under-illustrated, we certainly do not look on this as a defect. In fact we should consider the book to be improved if half the illustrations were removed and the space so saved utilised in the manner suggested later. After all, the really important things are the concise descriptions and the references to original sources of information. The illustrations, apart from their ornamental value, are merely useful for purposes of confirmation. The student who is interested in a particular disease will in any case have recourse to original publications and find the illustrations there.

The majority of the illustrations are good. There are a few (e.g. Fig. 35) of which this cannot be said, and the replacement of which in a later edition would be an improvement.

As the author states in his preface, the book is primarily intended for students and research workers in plant pathology. Considered as a guide to the systematic and field aspects of the subject, it is everything that could be desired. But, from the point of view of the person who is equipping himself for plant pathological work, it is lacking in one respect. There is no adequate treatment of methods of studying plant diseases, e.g. methods of isolating and culturing fungi, of artificial inoculations, of experimental field technique, etc. It is true that methods can only be thoroughly taught by oral and practical instruction. Nevertheless, we consider that a chapter dealing with methods of study would increase the usefulness of the book.

Again, we think that the conciseness which is so characteristic of this book and which is so admirable in the systematic descriptions, has perhaps been carried too far in the introductory chapter. We should like to see that chapter expanded to three or four times its present size. Even the adept in the science will be all the better for having the general principles and theories displayed with fitting elaborateness.

Reverting again to the preface, we note that the author expresses a hope that the book will be useful to the general botanist and to the practical grower "who takes an enlightened interest in his crops." We cannot see that this hope will be realised. We cannot see in fact that any one book can serve three purposes so diverse. The general botanist, who wishes merely to know what the plant pathologist is trying to do, and how he is doing it, would ask for the kind of elaborated introductory chapter of which we spoke above, together with perhaps a limited number of type diseases described in some detail. The practical grower, whose interest is primarily to avoid the disease, would insist on a change of view-point. To begin with he would want a rearrangement of the text on the basis of crop plants, and in the descriptions he would expect to read more about the crop and less about the fungus. In many cases, too, he would be disappointed with the sketchy accounts of control measures. We do not make these remarks by way of disparagement. As we have said before, the book is written for one main purpose and cannot meet three very different needs. Perhaps these remarks will serve a useful purpose in inducing Mr Brooks to take up his pen again and write the companion volumes.

. W. B.

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PHOMA RADICIS CALLUNAE

A PHYSIOLOGICAL STUDY

By M. C. RAYNER, D.Sc. & M. LLEWELLYN SMITH, M.Sc.

(With Plate VI and 4 graphs in the text)

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INTRODUCTORY

THE endophyte of *Calluna* was isolated by one of us (M. C. R.) in 1914 by applying the knowledge that seeds became infected externally by the endophyte while still enclosed within the fruits. Removed with aseptic precautions from unopened fruits and planted upon suitable media, seeds gave rise to colonies of non-sporing mycelium from which, on sub-culturing, pure cultures of the endophyte were obtained. A general account of this fungus and its behaviour in pure culture, with critical proof of its identity has already been published (Rayner, 1915).

In 1907 Ternetz had isolated five pycnidia-bearing fungi showing the general features of the genus *Phoma* from the roots of five ericaceous species, namely, *Oxycoccus palustris*, *Andromeda polifolia*, *Vaccinium Vitis-Idaea*, *Erica Tetralix* and *E. carnea*. These differed from all known species of *Phoma* previously recorded as associated with Ericaceae in the extremely small size of the pycnidiospores, $5\mu-4\mu$ long by $2.6\mu-2\mu$ broad, and were accordingly placed in a new species, *P. radicis*, by Lindau and Hennings (Ternetz, 1907). At the time their identity with any known species of *Phoma* was regarded as improbable and no fresh evidence has appeared since that this is the case.

Although included in a single species, the individual endophytes were characterised by definite morphological and physiological characters, a fact of some interest in view of frequent close proximity of roots of the different ericaceous species in their natural habitat.

In the case of the form now under consideration a high degree of specialisation to the symbiotic habit is known to accompany such differences. Individual endophytes are closely adapted to their hosts and are not interchangeable in respect to their symbiotic activities: that of *Calluna*, for example, cannot be used to induce germination of seeds of *Erica cinerea*, or of *E. Tetralix*. Whether this specialisation is absolute has not been ascertained.

Prolonged but unsuccessful efforts were made by Ternetz to raise seedlings of the various ericaceous hosts free from fungus infection; for this reason critical proof of the identity of any of the new forms of *Phoma* with the respective endophytes was not forthcoming until 1915 when it was established independently for that associated with *Calluna vulgaris* by isolation of an identical fungus from unopened fruits and re-inoculation into "pure culture" seedlings (Rayner, 1915). This endophyte had been isolated by Ternetz from roots of *Calluna* but was believed to be identical with that obtained from *Erica carnea* and was maintained in culture for a short time only¹. In view of its morphological identity with the fungus isolated from fruits in 1914, the name *Phoma radicis Callunae* was accepted for the latter on grounds of priority² (Rayner, 1922).

The work of which the present paper is an account was begun with a definite point of view and as part of a larger investigation.

In 1911 a research was undertaken to discover the biological

¹ Personal communication from Dr C. Ternetz to one of the authors.

² The characters of the pycnidia and size of spores in this fungus agree with those of *P. r. Ericae* described by Ternetz.

significance of the calcifuge habit in Ling, an enquiry that led speedily and inevitably to a closer investigation of the symbiotic relation between this species and its mycorrhizal fungus. The discovery of an association much more intimate than had been suspected widened the scope of the work and strengthened the opinion already formed that the edaphic peculiarities of ericaceous species are inextricably linked with the activities of their fungus associates (Rayner and Jones, 1911).

The so-called "calcifuge" habit is not, despite its misleadingly simple designation, a straightforward reaction to the calcareous content of soil. It offers an extremely complex problem, the elucidation of which in Ericaceae—and probably in all groups showing it—calls for more knowledge than is yet available of the metabolism of peat plants in general and ericaceous species in particular.

On a basis of experimental work carried on since 1911 and still in part unpublished, certain aspects have assumed special importance. In species such as *Calluna* may be mentioned: (i) the oil metabolism of the root cells; (ii) the reaction to hydrogen-ion concentration and salt content of the soil solution, and in particular, the response shown by the plant to interaction of these two sets of factors; (iii) the assimilation of nitrogen; interwoven with all such being the metabolic activities of the fungus endophyte that pervades the tissues.

ISOLATION OF THE ENDOPHYTE

Isolation of the endophyte of *Calluna* offered an opportunity for studying the reaction of the mycelium to various organic compounds in artificial culture and it was with the hope of contributing to the larger problem that the work now described was undertaken. It is still incomplete, but the results are not without significance in relation to those aspects of the problem just noted and it is hoped may eventually fall into place in a completed investigation.

Subsequently, the endophyte was several times re-isolated from fruits, the present account relating to a strain extracted in the autumn of 1923. Certain difficulties have always been encountered, e.g. seeds plated upon a suitable medium in the way described may show no signs of fungal growth, *although the presence of typical mycelium in the fruits from which they were taken be confirmed simultaneously by microscopic examination.* In order to secure a satisfactory technique, careful and repeated observations have been made on the distribution of mycelium within the fruits both in time and space, on the most suitable methods for external sterilisation of

fruits, and on the capacity for growth of the contained mycelium. From these observations the following facts have been established.

(a) *Mode of occurrence of mycelium in fruits*

Mycelium is present in the fruit cavities and associated with the seed-coats. It is usually very sparsely developed, but varies in amount in individual fruits; in general, it becomes relatively more abundant as these mature, and may sometimes be recognised without difficulty in the later stages of ripening. The very fine colourless hyphae are best observed by removing seeds and placentae from unopened fruits and mounting immediately in lactic-phenol.

(b) *Method of obtaining a pure culture*

Isolation of the endophyte is most readily effected by using ripe or nearly ripe fruits. At this stage, slight desiccation causes separation of the valves, for which reason capsules should be used as soon as possible after collection, and should be kept in a moist atmosphere during transit. Individual fruits must be examined carefully with a lens in order to ensure that those selected for sterilisation show no signs of splitting. This precaution is important because on dehiscence, however partial, the fruit cavity is subject to invasion by foreign organisms.

The external surface of the pericarp is not easily wetted by liquid disinfectants; moreover, the enclosed mycelium is readily injured by sterilising agents and great care is required in manipulation and in the selection of capsules into which the sterilising fluid cannot penetrate through slits formed during incipient dehiscence. Failure to obtain growth of mycelium from seeds or other contents of fruits in certain cases as noted above is now attributed to faulty technique in one or other of these respects.

The use of heat or alcohol as sterilising agents was found unsatisfactory. The method always adopted now is to immerse single fruits in 0·01 per cent. mercuric chloride solution for a minute or two, rinse several times in sterile water and dissect quickly on a flamed slide in a sterilised chamber, removing the seeds and contents of the ovary with a sterile needle, and avoiding contact with the outer surface of the fruit wall. Plate on any suitable agar medium and keep at laboratory temperature. As soon as a growth of mycelium from seeds or placentae is observed, sub-culture and keep under observation until pycnidia are produced. The commonest impurities are *Cladosporium* and *Alternaria*, species of which are frequent on

twigs and fruits of *Calluna*; if present in the same culture, mycelium of either of these fungi quickly overgrows that of the endophyte.

Using this technique, the endophytic fungus can be isolated with comparative ease and certainty during the period—varying in duration with the season and locality—when intact ripe fruits are available.

A. NUTRITION

Preliminary observations

In the earlier stages of the work, the behaviour of the endophyte was studied on a number of different media; included among these were *Calluna*-shoot-extract agar, *Calluna*-root-extract agar, potato agar, *Calluna*-shoot-extract gelatine, and various other nutrients.

Well-marked effects upon rate of growth, manner of growth, e.g. whether above or below the surface of the medium, sporing capacity, and also in respect to morphological differences in the hyphae were noted and related directly to variation in the sub-strata. For example, on *Calluna*-extract—whether of root or shoot—growth is less vigorous than on synthetic media containing small quantities (2 to 0.1 per cent.) of sugar or other organic materials; the character of the mycelium and its contents differed on root-extract and shoot-extract respectively. Sporing has never been observed when the mycelium is grown on *Calluna*-shoot-extract agar or gelatine without passage through another nutrient after extraction.

Very rarely, however, feeble sporing has been observed in a culture sub-cultured to *Calluna*-shoot-extract from a sporing colony growing on a medium favourable to pycnidia formation.

In order to maintain cultures in good condition over long periods, it was found desirable to sub-culture alternately to different media, a decrease in vigour as evidenced by progressively weaker growth of the colonies accompanied by permanent loss of sporing capacity, occurring when the organism was sub-cultured repeatedly on any of the synthetic media used. This matter is considered more fully in a subsequent paragraph and it may be mentioned that behaviour of a similar kind for a number of parasitic species of *Phoma* has been recorded by Westerdijk (1920).

It has been already noted that the form of *Phoma radicis* isolated from *Calluna* hydrolyses arbutin when growing in pure culture and under the same conditions rapidly liquefies gelatine, the rate at which a 15 per cent. gel in a solution of salts is liquefied at laboratory temperature being approximately twice as rapid without sugar as it is with the addition of 1 per cent. dextrose.

From the first the endophyte had been cultivated on *Calluna*-shoot-extract for purposes of inoculation into sterile seedlings. These earlier synthetic cultures were invariably successful, whereas in later work it was observed that inocula from other media containing organic nutrient usually parasitised the seedlings. It was also noted that growth of the mycelium on the shoot-extract medium became progressively weaker and finally failed to sub-culture, some constituent evidently exerting an inhibiting effect on growth and vitality. Ordinarily the endophyte does not form pycnidia on agar or gelatine media prepared from shoot-extract.

In view of these facts and of the markedly reduced vigour of endophytic mycelium in shoot tissues of *Calluna* as compared with those of roots it was thought desirable to observe the behaviour of the fungus on artificial media prepared from root-extract and shoot-extract respectively¹.

In all such comparative cultures growth was slower on shoot extract with conspicuous differences in respect to the size and contents of the hyphae. Corresponding cultures using *P. betae* gave a similar result with decreased growth in both cases, whereas, on all other media tried, *P. betae* grows more vigorously than *P. radicis*.

It may be concluded therefore that extract of *Calluna* shoot slightly favours growth of the endophyte as compared with certain parasitic species of *Phoma* but that, in the concentration used, it exerts an inhibitory effect on growth that becomes lethal in successive sub-cultures. Cultures on root-extract do not suffer in this way; they retain their vitality for an indefinite period and have given vigorous sub-cultures after four to six months.

Comparative cultures of *P. r. Callunae* and *P. betae* on synthetic media containing dextrose and peptone gave similar types of sporing colonies with more marked browning of the medium by the last-named species.

CULTURES IN AGAR MEDIA

The discovery of regular and extensive digestion of mycelium in the root-cells of *Calluna* attracted attention to the metabolism of the fungus in relation to organic substances likely to be present in the root-cells and the possibility of relating this in some way with the edaphic peculiarities of the host plant. The presence of much

¹ The extracts were prepared as follows: 200 grams fresh weight of young shoots or roots respectively, extracted by autoclaving in 1000 c.c. of conductivity water.

fatty material in these cells had been noted and the recent observations of Hinchliff and Priestley (1924) are of special interest in this connection. As a preliminary step and in order to test the reaction of the endophyte to various carbon compounds, a series of culture media was devised containing respectively *Calluna* oil, starch, dextrose and saccharose in the same proportions.

The oil used was extracted from ripe seeds, the average fat content of which amounts to 42.5 per cent. of the dry weight. After extraction in hot chloroform and distillation under reduced pressure, the fatty contents of the seeds yield a golden brown oil which oxidises to a firm elastic varnish on exposure to air. The freshly extracted oil was added to a solution of salts and mixed by prolonged shaking in a mechanical shaker. This treatment gave a homogeneous emulsion from which the oil did not separate after addition of agar and sterilisation. The salts used were the purest obtainable and the solutions were made up with freshly distilled conductivity water.

Salt solution A:

KNO ₃	1	gram.	NaCl	0.5	gram.	
MgSO ₄	0.4	"	FeCl ₃		Trace	
CaSO ₄	0.5	"	Water		1000 c.c.	
CaH ₄ P ₂ O ₈	0.5	"	Agar-agar		15	gram.

Salt solution B:

KCl	0.5 gram.	NaCl	0.5 gram.
MgSO ₄	0.4 "	FeCl ₃	Trace
CaSO ₄	0.5 "	Water	1000 c.c.
CaH ₄ P ₂ O ₈	0.5 "	Agar-agar	15 gram.

Series 1:

1. Solution A plus 2 % *Calluna* oil (N as nitrate)
2. Solution B " " plus 1 % Witte's peptone (N as peptone)
3. " " " (lacking combined N)

Series 2:

1. Solution A plus 2 % pure potato starch (N as nitrate)
2. Solution B " " " plus 1 % Witte's peptone (N as peptone)
3. " " " (lacking combined N)

Series 3:

1. Solution A plus 2 % saccharose (N as nitrate)
2. Solution B " " " plus 1 % Witte's peptone (N as peptone)
3. " " " (lacking combined N)

Series 4:

1. Solution A plus 2 % dextrose (N as nitrate)
2. Solution B " " " plus 1 % Witte's peptone (N as peptone)
3. " " " (lacking combined N)

The cultures were grown in small flasks, each containing 25 c.c. of medium and were kept under constant conditions of light and temperature. Sterilisation was effected by thrice repeated steaming, autoclaving being regarded as undesirable in view of the constitution of the media. Each of the four series was triplicated in relation to its nitrogenous content (see Table, p. 267). All culture flasks were kept for six days before inoculation, after which four parallel groups were inoculated from cultures of different ages and histories, so that the total number of cultures included in the experiment was 48.

The inocula for Group 1 were obtained from a 5-day culture on potato agar, sub-cultured from an 83-day culture on *Calluna*-root-extract direct from isolation. On root-extract the mycelium grew entirely below the surface and never formed pycnidia; transferred to potato-agar, it produced vigorous aerial mycelium and spored freely. For Group 2 the inocula were provided by a 5-day culture on dextrose-peptone-agar, sub-cultured from the same medium on which it had been grown continuously from isolation. Inocula for Group 3 were taken from an old culture (100 days) on *Calluna*-root-extract on which the mycelium had been grown continuously from isolation. For Group 4 an original colony on *Calluna*-shoot-agar derived directly from mycelium present in unopened fruits without sub-culturing was used.

Observations were made upon rate of growth as estimated by measurement of the diameter of the colonies, upon sporing capacity, and, at the end of six weeks' growth, upon chemical changes in the media. Deductions from such of these as relate to sporing are rendered unsafe by the facts described under "sectoring" in a later section of the present paper (see p. 282).

The preliminary observations on chemical changes produced during six weeks' growth may be summarised as follows.

(a) *Change of hydrogen-ion concentration*

Earlier observations had shown that the endophyte could be cultivated on peat extract¹, making a profuse superficial growth of mycelium very similar to that associated with roots.

Growth brought about a change in reaction in this medium from an initial pH value of 5.2 to one of 7.0-7.1 after 58 days' growth.

Of the cultures now under consideration, all, whatever the foodstuff provided, showed a marked increase in alkalinity in the substrate.

¹ 200 grm. fibrous peat extracted by autoclaving in 1 litre of conductivity water.

The initial pH values and those after six weeks' growth are recorded in Table I, reference to which shows that the rise in pH value reached a maximum in the cultures supplied with nitrate and was least, although well-marked and constant, in those lacking combined nitrogen.

From scrutiny of the figures it is evident that the values noted after three months' growth represent the highest that can be reached under the experimental limitations existing in the cultures. It was regarded as significant that the change in reaction was similar in kind in all flasks, i.e. in those lacking combined nitrogen as well as in those supplied with nitrates or peptone. Possible causes of increased alkalinity are: (i) the production of ammonia in the course of nitrogen metabolism, or (ii) of bicarbonate as an indirect result of the accumulation of CO_2 . At the end of six weeks no ammonia could be detected in any of the cultures and at this stage the matter was not more fully investigated (see p. 281).

(b) *Reaction to various sources of carbon*

Active growth took place in all four series. As measured by increase in diameter of colonies no marked differences in rate or vigour of growth could be related to the presence of oil, starch, or sugars. There seems to be no doubt, therefore, that *Calluna* oil, starch, dextrose, and saccharose can be utilised with equal ease by the endophyte as sources of carbon. During six weeks' growth all the starch, dextrose and saccharose provided in 25 c.c. of the various media were used up, and, in those cultures supplied with nitrate, this also disappeared; in some of the oil cultures, the medium still gave a brownish coloration with 1 per cent. osmic acid; in all of them the osmic acid test supplied positive evidence of the presence of fatty or oily material in the hyphae. All the culture media, whatever the source of carbon provided, after six weeks' growth gave a colour reaction with iodine similar to that produced by dextrin (see p. 282).

Marked differences of behaviour in respect to rate of growth, colour changes in the medium, and sporing capacity were observable in the different groups as compared with one another although not among members of any one series *inter se*.

(c) *Reaction to various sources of nitrogen*

No constant differences were observed in the two sets of cultures supplied with combined nitrogen in the forms respectively of nitrate

and peptone except in respect to the higher pH values observed in the former; in both, vegetative growth was vigorous and spores were freely produced in those series which formed pycnidia. The cultures on media lacking combined nitrogen grew at the same rate as measured by diameter of the colonies but pycnidia were produced later and, in most cases in less profusion than in those supplied with nitrate or peptone. The lag in development noted in these cultures tended to disappear with age; growth became more vigorous and the capacity to form pycnidia increased. As tested by variation in pH values the chemical changes produced in the media lacking combined nitrogen were similar in kind to, but less in degree than, those taking place in the others. After six weeks' growth no traces of ammonia were detectable in any of the cultures (see p. 281).

(d) *Sporing capacity and "staling"*

In any one group, i.e. in one containing only cultures of the same age and of similar origin, no differential effect in respect to sporing capacity could be related directly to the nature of the carbon supply. Whether this is equally true in respect to nitrogen is not certain; it is possible that the constitution of the media, in respect to nitrogenous material or the value of the carbon/nitrogen ratios may prove to be important in relation to chemical changes produced during growth, the latter in turn being responsible for the phenomena about to be described. As already noted, continuous culture of the endophyte on the same nutrient leads to complete loss of sporing capacity. At first, change of food produces a temporary restoration of the ability to form pycnidia and spores, but the physiological change manifested as loss of sporing capacity is apparently irreversible if the conditions inducing it are long continued. It is doubtless for this reason that the behaviour of cultures in this respect in any one group was found to depend rather upon the age and history of the culture used for the inoculum than upon the nature of the food material provided. Thus, in those now under consideration: in Group 1, pycnidia were forming in great numbers in all flasks at five days from inoculation (with sectoring); in Group 3 at 12 days (with sectoring); in Group 4 pycnidia were still absent after 20 days and were but scantily produced later in a few flasks; while in Group 2 sporing was restricted to one small group of pycnidia in the oil medium *lacking combined nitrogen*.

As at present observed, loss of sporing capacity may occur in cultures of any age and appears to be definitely related to growth

changes. It is believed to be a definite character of the organism existing independently of the phenomena described under "Sectoring," perhaps underlying them. The cause is at present unknown. The term "staling" may be used to describe it without reference to its meaning as used by other workers or in relation to other fungi. The observations now recorded agree with and amplify those of Westerdijk (1920) on a number of parasitic species of *Phoma* in laboratory culture, and raise points of interest in connection with the endophytic habit of *Phoma radicis* (Pl. VI, fig. 1).

In *Phoma radicis* the "staling" effect of age is manifested first by marked irregularities of sporing capacity quickly followed by complete disappearance of the power to form pycnidia or spores.

As used here, "age" relates to the length of time during which the mycelium has been cultivated outside the host rather than to duration of individual cultures. These retain their vitality unimpaired over long periods and have been used for sub-culturing after many months without giving any sign of reduced vegetative vigour or sporing capacity *if still in the sporing phase*. For example, inocula from cultures of similar history aged 8, 75 and 100 days respectively gave daughter colonies that spored profusely from the two last and a colony that formed but few pycnidia from the 8-day culture. On the other hand, after continuous sub-culturing for 6 to 12 months, it is apparent that the mycelium suffers physiological change expressed by complete loss of sporing capacity.

Pycnidia are never produced when the mycelium is associated with roots of *Calluna* in normal mycorrhiza, but they have been observed in the abnormal condition induced experimentally by an alkaline rooting medium (Rayner, 1915). The exact significance of the fluctuations in sporing capacity is obscured by lack of knowledge of the causes underlying "sectoring" a phenomenon first recognised in the cultures under discussion. The possibilities thus introduced are discussed in a later section (p. 282). It is believed, however, that irregularities in respect to sporing are related to the symbiotic habit of the organism in nature, and it is not without interest in this connection that the host occurs almost invariably in relatively large vegetative units and that there is at present no record of a free-living soil *Phoma* with characters resembling those of *P. radicis*. Recently, attempts have been made to restore the capacity for pycnidium formation by exposure to radiation from a mercury vapour lamp in the manner described by Stevens (1928). Direct exposures varying in duration from 30 seconds to 3 hours under conditions

comparable with those employed by Stevens evoked no response from *Phoma radicis Callunae* in respect to spore formation. More remarkable, not only did the shorter exposures produce no lethal effect whatever, but even those of one hour and longer only caused injury to the aerial mycelium soon masked by a rapid growth of new hyphae from that in the thin layer of agar below.

(e) *Oxidase reactions*

The mycelial mat gives a strong reaction for oxidase or peroxidase with benzidine in 30 per cent. alcohol. In general, this is strongest in cultures containing nitrate and in those lacking combined nitrogen, weak or absent in those supplied with peptone; the most marked reactions were given by mycelium in cultures supplied with *Calluna*-oil and peptone, *Calluna*-oil lacking combined nitrogen, starch and nitrate, and glucose lacking combined nitrogen. No reaction for oxidase or peroxidase was given by the cultures supplied with *Calluna*-oil and nitrate.

Since a thorough analysis could not be undertaken at the time, the cultures and samples of the original media were preserved by the addition of absolute alcohol, corked securely and waxed over the corks. Three years later the chemical analyses were repeated and the work extended by the investigation of fresh cultures on similar lines. The methods employed included: re-analysis of the original agar cultures, and, in new series of cultures in liquid media, comparative estimation of dry weights of mycelia, observation of changes in hydrogen-ion concentration, and investigation of the course of carbon and nitrogen metabolism.

In respect to the original cultures, the results obtained were substantially the same as those just summarised and are tabulated in Table II. It was, however, regarded as so surprising that the cultures to which *Calluna*-oil or sugar was supplied should produce dextrin that this matter was further investigated.

CULTURES IN LIQUID MEDIA

Attention was turned to solutions of agar in distilled water and it was found that all samples tested gave a dark reddish coloration with iodine, thus obscuring the normal reaction for dextrin. Culture media containing agar are inconvenient also for purposes of chemical analysis inasmuch as it is necessary to dialyse the solutions before testing for non-reducing sugars. It was decided therefore to set up a duplicate set of cultures in liquid media so avoiding the diffi-

culties caused by the use of agar. The solutions used were similar with the exception that the *Calluna*-oil cultures were not repeated on a liquid substratum.

Although *Calluna*-oil was not available a set of cultures was grown in oil extracted from seeds of *Vaccinium macrocarpum*. The observations on these cultures are included in Table II and in the graphs and are of some interest as showing that this oil was utilised (as are also olive and linseed oils). The figures recorded have no comparative value because oxidative changes due to age rendered it impossible to obtain a homogeneous oil emulsion.

In one other respect these cultures are not strictly comparable with those on the original agar media. The strain of mycelium used had been cultivated outside the host plant for between three and four years. Although still relatively vigorous it had evidently suffered physiological change as evidenced by loss of sporing capacity. That changes affecting the course of metabolism do occur as soon as the endophyte is cultivated on synthetic media is certain and is proved, not only by the observed loss of sporing capacity, but also by the extreme care that must be exercised in the selection of media on which to cultivate mycelium before bringing it into contact with pure culture seedlings.

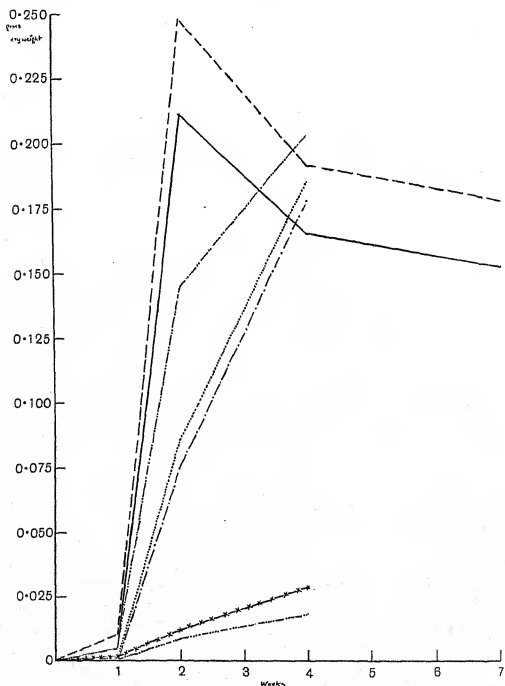
The cultures were set up in triplicate, flasks being withdrawn for analysis after 7, 14, 28 days' and 7 weeks' growth. The solution was poured in 25 c.c. quantities into small conical flasks, sterilised by repeated steaming and inoculated from a culture of the endophyte on malt agar. Uninoculated controls were kept in the same incubator throughout the growth period and analysed simultaneously with the cultures. The results of analysis are summarised in Table II and will now be discussed.

(a) *Dry weight of mycelium*

The increase in dry weight of the various cultures during growth is plotted in Graph I. Examination of this shows that the more rapidly growing cultures increased in dry weight up to the 14th day after which respiration overpowers assimilation and the dry weight diminishes. In the more slowly growing cultures the maximum weight was not reached until after 28 days' growth. Comparatively, those supplied with glucose make more weight than those supplied with starch. Inasmuch as the loss of dry weight in peptone cultures during the two weeks from the 14th to the 28th day of growth is nearly three times as great as that during the three weeks from the 28th to the

49th day of growth, it is evident that the limits of normal growth under the conditions supplied are reached by the 3rd week.

As regards nitrogenous food supply, nitrate cultures grew slightly less rapidly than those supplied with peptone; those lacking com-



Graph I. Showing dry weight changes during growth.

- | | |
|------------------------------------|----------------------------------|
| — starch-peptone | ----- glucose-peptone |
| <i>Vaccinium</i> oil-peptone | glucose-nitrate |
| —+— starch-nitrate | — x — x — glucose minus nitrogen |
| starch minus nitrogen | |

bined nitrogen showed a marked lag in growth and had not reached their maximum dry weight at 28 days when the last analyses were made. These dry weight estimations confirm the accepted view that values based on superficial measurements of fungus colonies are not reliable as comparative indices of growth.

The claim for nitrogen fixation by this fungus has recently been made the subject of a special research and no special attention was given to this matter in the cultures now under consideration (Neilson Jones and Llewellyn Smith, 1928).

As regards manner of growth, at first the mycelium grew below the surface; later, it showed profuse aerial growth in all media except those lacking combined nitrogen. No pycnidia were formed and it was known before the cultures were started that the strain used had lost the capacity for forming pycnidia after cultivation for four years outside the host plant. Peptone cultures showed a considerable darkening of the medium during growth. In all cases but one the mycelium remained colourless throughout the growth period; in one culture on glucose-nitrate it developed a pinkish tinge, a similar coloration having been observed in mycelium grown on malt-agar. The incubator in which the cultures were grown smelt rancid during the earlier stages of growth due doubtless to the production of fatty acids; this odour disappeared later.

(b) Carbon metabolism

The starch-nitrate cultures showed a regular conversion of starch to dextrin and of the latter to reducing sugar that, in turn, disappears. In the starch-peptone cultures, the dextrin formed persists throughout the growth period, a possible explanation of this being the fact that amylase is not active at high pH values. The peptone cultures had a slightly higher initial value and after two weeks' growth had reached a pH value of 7.5 when the starch-nitrate cultures were still at 6.9. Dextrin disappeared from the nitrate cultures during the second fortnight of growth. In the third week the average pH value of the peptone cultures was about 7.6 and of the nitrate cultures about 7.2. If the upper limit for amylase activity in *Phoma radicis* is assumed to be about 7.5, the peptone cultures which reached this value before hydrolysis of all the dextrin produced would continue to give positive dextrin reactions, whereas the less alkaline nitrate cultures would cease to give this reaction after the third week of growth, a hypothesis fitting the observed facts.

In cultures supplied with glucose instead of starch the sugar is apparently used directly, no other carbohydrates being detectable at any time (i.e. the positive dextrin reaction observed in the original agar cultures was due to partial hydrolysis of the agar). No oxalates or carbonates were detected in any of the cultures at any stage of growth.

In the more slowly growing cultures lacking combined nitrogen, starch is present and the mycelium still growing at the end of 28 days; glucose and dextrin are produced and hydrolysis appears to proceed normally as in nitrate cultures.

(c) *Production of ammonia in relation to changes of reaction*

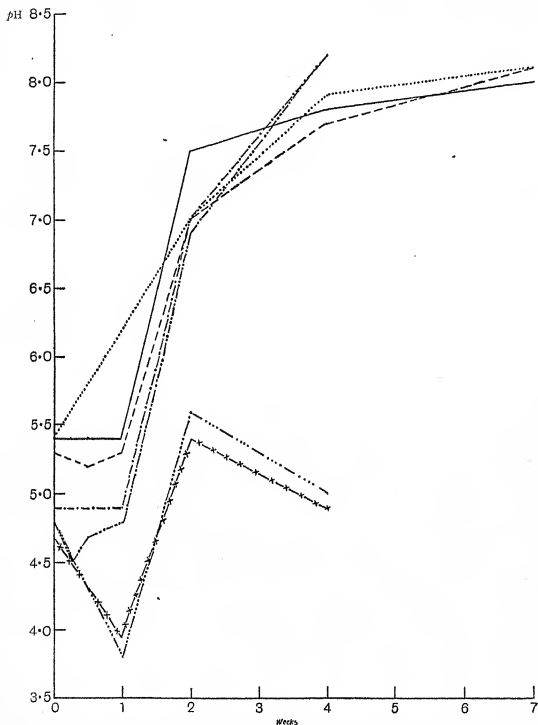
In cultures supplied with combined nitrogen the pH values rose steadily to about 8.0; in those lacking combined nitrogen the value at first declined, i.e. the media became more acid, and then rose to a figure somewhat above the initial value.

The possibility of an initial increase of acidity in nitrate and peptone cultures, overlooked owing to their much more rapid growth, was investigated by a series of special cultures in which the pH was measured 2, 4 and 7 days from inoculation. A slight drop of pH value was in fact observed and it was concluded that the greater persistence of this feature in cultures lacking combined nitrogen is directly related to the marked "lag" in growth.

The changes in pH value observed in cultures in liquid media are recorded in Graph II. Let us consider the rise and fall in hydrogen-ion concentration observed in all these cultures in connection with the possible production of fatty acids and ammonia.

In peptone cultures ammonia was found in considerable quantity as growth proceeded and could be detected by its odour when the solution was boiled as well as by the Nessler test. In nitrate cultures the amount of ammonia produced was smaller while in those lacking combined nitrogen no detectable quantity was found. In nitrate cultures no fatty acids accumulated nor were carbonates formed at any time. It seems therefore that the rise in pH must be due at least in part to ammonia production. It may be asked why then is the rise in pH in the cultures containing peptone not far greater than in those containing nitrate since the amount of ammonia produced in the former is larger? But, in the first stages of growth, considerable amounts of fatty acids were formed in these peptone cultures as proved by the odour of the solution and by other reactions.

These acids subsequently disappear, being doubtless oxidised to carbon dioxide that escapes from the still acid solution. If ammonia



Graph II. Showing changes in reaction during growth of cultures.

- | | |
|------------------------------------|----------------------------------|
| — starch-peptone | ----- glucose-peptone |
| <i>Vaccinium</i> oil-peptone | — · — · — glucose-nitrate |
| — · — · — starch-nitrate | — x — x — glucose minus nitrogen |
| ----- starch minus nitrogen | |

were produced simultaneously, the products would be ammonium salts of the fatty acids none of which would affect the pH value appreciably. Only when the amount of ammonia exceeds that of the diminishing fatty acids would the increasing alkalinity become evident by a rise in pH value. Moreover, when the pH value reaches a high figure, ammonia tends to escape as may be confirmed in bacterial cultures of pH 8-9 where the escaping ammonia can be identified by the litmus reaction.

It follows that the rise of pH value in peptone cultures is limited, evolution of ammonia taking place after a certain critical value is reached, the actual amount of ammonia produced previously being regulated by the quantity of fatty acids present.

In the cultures lacking combined nitrogen no ammonia was detected. At first the pH value falls owing to the production of fatty acids; later, these disappear owing to oxidation and the pH rises above its original value. Whether the increased alkalinity is due to differential absorption of inorganic ions from the solution or to some other cause is not known and requires further investigation.

It was believed that titration curves of the media against standard ammonia plotted over the range of pH values observed during growth might yield information as to the amount of ammonia produced by the fungus in nitrate and peptone cultures. Assuming rise of pH value to be entirely due to ammonia it should be possible to read off from the curves the amount required in order to produce a given rise in pH value. Moreover, in the case of cultures not showing an initial drop in pH value, this assumption is justified, since oxidation of fatty acids produced during growth could not depress the hydrogen-ion concentration below its original value. If, therefore, we measure the rise in pH value from the start of growth and disregard any fall that may have occurred, we obtain a minimum value for the amount of ammonia produced during growth.

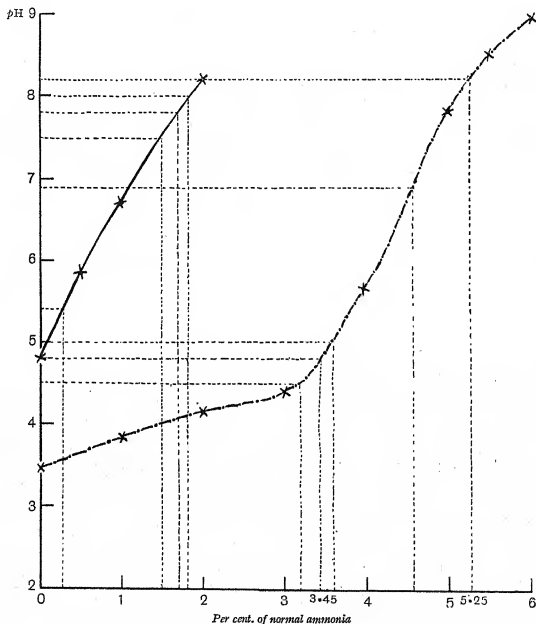
For cultures that showed an initial lowering of pH value, a maximum figure for the amount of ammonia produced can be found by measuring the rise in pH from its minimum instead of its initial value.

(d) Amounts of ammonia calculated from titration curves

The titration curves shown in Graphs III and IV were obtained as follows:

A solution of normal ammonia was made up and standardised against normal hydrochloric acid. The culture medium was placed

in 10 c.c. quantities in test tubes to which varying amounts of standard ammonia were added by means of a graduated 1 c.c. pipette. The pH value was measured electrometrically using a quinhydrone electrode.



Graph III. Titration curves of starch culture media with ammonia.

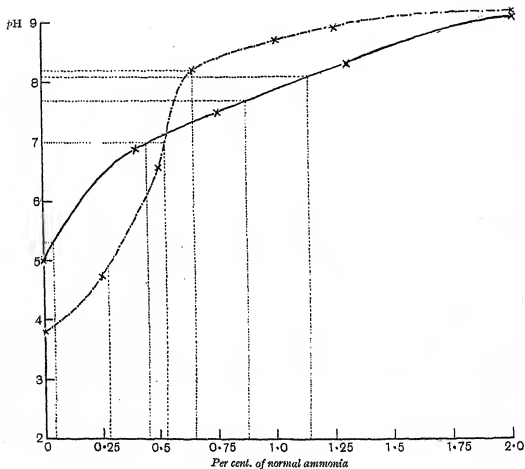
— starch-peptone

- - - starch-nitrate

From these curves can be read off the percentage of normal ammonia needed to raise the pH value of the medium by the required amount, whence can be calculated the amount of nitrogen present in 25 c.c. of the culture medium at different times during

growth, assuming that the rise in pH is entirely due to ammonia. For example, the quantity of ammonia produced in a starch-nitrate culture after four weeks' growth can be found as follows:

Initial	pH value	4.8,
Minimum	"	4.5,
Final	"	8.2.



Graph IV. Titration curves of glucose culture media with ammonia.
 — glucose-peptone - - - glucose-nitrate

From the curve (Graph III) the amount of normal ammonia required to raise the pH value of the medium from 4.8 to 8.2 is 5.25-3.45 or 1.80 per cent. Hence, in 25 c.c. of medium the minimum amount of ammonia produced during four weeks' growth is

$$\frac{1.8 \times 25}{100} \text{ c.c.} = 0.45 \text{ c.c.,}$$

and this volume of normal ammonia contains

$$\frac{1.8 \times 25 \times 0.014}{100} = 0.0063 \text{ gram. of nitrogen.}$$

The amount of ammonia needed to raise the pH value from 4.5 to 8.2 represents the maximum amount of ammonia produced during growth. Thus, from the curve 5.25-3.20 c.c. = 2.05 c.c., i.e. in 25 c.c. of solution there are

$$\frac{2.05 \times 25 \times 0.014}{100} = 0.0072 \text{ gm. of N as ammonia.}$$

Therefore, at the end of four weeks' growth, the ammonia content of a starch-nitrate culture would be such as to contain between 0.0063 gm. and 0.0072 gm. of nitrogen.

A less complicated method of estimating the ammonia present directly by means of Nessler's solution was not applicable to these cultures because the results were obtained only after four weeks' growth and could not have been predicted; only 25 c.c. of culture fluid was available and this had to be used for other analyses; the titration method used has the advantage that curves can be obtained from the stock medium at any time.

From these figures it will be seen that limiting values can be set to the amounts of nitrogen present as ammonia although it is not possible to calculate the exact figures. More ammoniacal nitrogen is produced in the starch cultures than in the corresponding glucose cultures. In those containing peptone, the ammonia is probably due to hydrolysis of peptone; in the nitrate cultures it appears to be formed by reduction of nitrate.

When the original agar cultures were under observation, it was believed, in view of their composition, that the rise in pH value would be found to be due to the production of ammonia. No trace of ammonia or ammonium salts was found, however, in any of these cultures at the end of six weeks' growth. In view of the results of the analyses of cultures in liquid media just cited it is believed that this negative result was entirely due to the age of the cultures. If, as appears to be the case, effective growth ceases after about three weeks in cultures supplied with 25 c.c. of medium (see Table II), the lapse of several weeks subsequently would afford ample time for the disappearance of any free ammonia present (see p. 269).

(e) *Fixation of atmospheric nitrogen*

There remain for consideration the cultures grown without a supply of combined nitrogen. As has been already stated, no ammonia was detected in these at any time; the rise in pH above the initial value had not reached its maximum value when the cultures

were brought to an end and its cause remains unexplained. It is of interest that the mycelium grew well lacking a supply of combined nitrogen, increasing considerably in dry weight although not nearly so rapidly as those supplied with nitrogen. From earlier work on the corresponding agar cultures it is apparent that their maximum development was reached only after 5-6 weeks' growth. The nitrogen necessary to maintain growth over such a long period, since it was not supplied, must have come from the atmosphere. No special precautions were taken to protect the cultures from ammonia or other gaseous nitrogen compounds that might be present in the air. It is not however likely that such contaminants would be present in significant amounts though such a possibility must be considered. It is suggested that the healthy growth of cultures containing no combined nitrogen over periods of six weeks and more provides additional evidence of the nitrogen fixing capacity of *Phoma radicis*.

Being already the subject of a special research the matter was not investigated further (Neilson Jones and Llewellyn Smith, 1928).

B. SECTORING

In one respect the earlier agar cultures described in this paper were remarkable. At a relatively early stage of growth, individual colonies on various media exhibited the phenomenon known as sectoring. The appearance of colonies showing this diagrammatic sectorial growth is illustrated by the accompanying photographs, sectoring being determined by the presence of pycnidia in certain sharply defined areas of the plate and their absence from others, the former coinciding in all cases with regions of more vigorous vegetative growth (Pl. VI, figs. 2, 3, 4).

Inasmuch as a result of this kind might conceivably arise in a circular colony containing a mixture of strains, sporing and non-sporing respectively, the possibility of such a constitution in the original inoculum was investigated by means of sub-cultures from sporing and non-sporing areas. In every case the differential effect persisted through a number of generations, thus lending support to the view that hyphae of different constitution and different potentialities are actually present in each colony that exhibits the phenomenon of sectoring. The result of sub-culturing from sporing and non-sporing regions of a single culture of this kind for five generations over a period of five months is shown in the accompanying photographs (Pl. VI, figs. 5, 6, 7, 8).

During this time the capacity for forming pycnidia steadily decreased in the sporing strains. It then disappeared as in the original cultures and was not revived either by change of medium or by bringing together the sporing and non-sporing strains. Both strains have now been in cultivation for four years without again showing any sign of pycnidium formation. More vigorous growth characterised by a greater development of aerial mycelium by the sporing strains has been maintained, but the general vigour of both strains is reduced as compared with that of the original mycelium on similar media immediately after isolation. In tube cultures, minor inconsistencies of behaviour, e.g. the appearance of mycelium of pinkish colour have been noted; otherwise the mycelium of the endophyte has maintained its original morphological characters.

Assuming that the phenomena described do actually depend upon the existence of distinct strains of the fungus, two possible explanations may be put forward:

(1) that hyphae of two strains, sporing and non-sporing respectively, are present in the inoculum used for each of the sectoring cultures; or,

(2) that such strains arise independently as "sports" or saltations during the growth of many independent cultures on different substrata.

On either of these hypotheses, it might be expected that the mosaic pattern consists of (*a*) regions occupied by hyphae of the non-sporing strain only, and (*b*) regions occupied by hyphae of the sporing strain, with or without mixture of the hyphae of the non-sporing strain. On either view, however, it is necessary to assume the presence of both strains in the sporing sectors, since subsequent generations of colonies sub-cultured from those areas have been observed to repeat the phenomenon of sectoring. It is difficult to account for the sharp delimitation and symmetry of sporing and non-sporing areas in the sectoring plates.

The simplest interpretation of the evidence available is to regard sectoring as due to the sudden appearance of a non-sporing saltation, differing from the normal form by loss of sporing capacity. The sudden appearance of a variant in *Botrytis* sp., involving loss of colour in the sclerotium, has been put on record by Brierley (1921). Phenomena of a similar kind involving vegetative characters have also been described recently for a species of *Fusarium*. In the latter, the sudden appearance of sharply delimited sectorial areas in plate cultures mark the origin of a number of definite strains, each

differing in certain well marked mycelial characters from the parent colony, and retaining such character or characters when sub-cultured (Brown, 1924).

Before accepting this view, however, it must be noted that a number of very puzzling observations in respect to irregularities in sporing capacity had been made on *Phoma radicis* before the recognition of sectoring as a disturbing factor. For example, during the year following isolation of the fungus, the capacity to form pycnidia decreased or disappeared after repeated sub-culturing to the same medium, reappearing in full strength when a change of medium was supplied—the exact nature of the variation in nutrient seeming to be of secondary importance.

It is evident that any attempt to explain the significance of sectoring must take account of facts of this kind. It is clear also that the possibilities involved in the causes that underlie sectoring make it unsafe to base any general conclusions on the results of observations seeking to correlate growth and sporing capacity with differences in constitution of the various substrata used. Moreover, these possibilities illuminate, although they do not explain, earlier records of inconsistencies with regard to the sporing capacity of *P. r. Callunae*.

It may be noted that the ancestral culture from which all those exhibiting sectoring were ultimately derived was produced in the first instance from mycelium associated with a single seed. It must be assumed, therefore, that hyphae capable of giving rise to both sporing and non-sporing strains are present in a small group such as may be observed on an isolated seed.

Renewed study of the relation, if any, between the phenomena included under staling (i.e. loss of sporing capacity) and those responsible for the appearance of sectoring can best be undertaken on a series of single spore cultures. Unfortunately, this work was postponed until pycnidia were no longer available in the strain of *Phoma radicis* under consideration; attempts to revive sporing capacity have not been successful, and it must await the isolation of a fresh strain of the fungus from the host plant.

DISCUSSION

The presence of much fatty material in the mycorrhizal cells of *Calluna* points to oil as an important reserve substance, and the lack of more exact data from the earlier cultures supplied with *Calluna*-oil is especially regrettable in view of the failure to repeat them.

Mycelium of the endophyte grew very vigorously in these cultures and although dry weight estimations are unfortunately not available it is probable that the total growth equalled or exceeded that of those supplied with starch or sugar. Moreover, both in the root cells and in cultures supplied with oil, the mycelium becomes gorged with fatty material as shown by microchemical tests.

There can be little doubt that fatty acids are produced both in artificial cultures containing oil and in the root cells. Failure to obtain chemical evidence of their presence as also of ammonia—in the former—was evidently due to their age, since it has been shown that normal cultures supplied with 25 c.c. of medium reach maximum development in two to three weeks, thus allowing ample time for oxidation or neutralisation of fatty acids and disappearance of ammonia from six-week cultures.

The increased alkalinity observable in all cultures supplied with combined nitrogen is undoubtedly due to the production of ammonia overpowering an initial fall in pH value due to the production of fatty acids.

In view of the observed ability of the mycelium to liquefy gelatine in culture and utilise peptone as a source of nitrogen, it is reasonable to assume that corresponding processes occur in the root-cells and to infer that the resulting changes in hydrogen-ion concentration may not be without effect in determining the preference of the host plant for neutral or acid soils. The extraordinary development of bacterial colonies about the roots of *Calluna* seedlings in calcareous soil is of remarkable interest in the same connection (Rayner, 1913).

Strong oxidase or peroxidase reactions were given by the mycelial mat in many of the agar cultures. Indeed, pronounced oxidase activity appears to be characteristic of mycorrhiza in general. The mycorrhizal cells of *Calluna* in fresh roots give a strong oxidase reaction with benzidine in 30 per cent. alcohol, and in Conifers Laing (1927) has observed similar behaviour in the mycelial sheath of endotrophic mycorrhizas and within the tissues of those showing endotrophic infection. It appears therefore, as pointed out by Laing, that mycorrhizal plants have at their disposal a relatively large volume of oxidising agent of great potential value in certain habitats (Laing, 1927).

In general, in so far as study of the endophyte of *Calluna* in pure culture affords clues to its metabolic behaviour when growing symbiotically, the following features may be noted as of special interest:

(a) The possession of an efficient enzyme mechanism for hydrolysing proteins, as indicated by the rapid liquefaction of gelatine.

(b) The effectiveness of oils, especially of that extracted from seeds of *Calluna*, as a source of carbonaceous food material.

(c) The marked tendency to bring about an alkaline reaction in all media used, whether cultures were supplied with nitrogen in inorganic or organic forms or deprived of any source of combined nitrogen.

(d) The oxidising activity of the mycelium.

Some of these clues may point the way to a better understanding of the edaphic peculiarities of ericaceous species in respect to the preference shown by many of them for an acid rooting medium and hence to the origin of the calcifuge habit. The phenomena are of such importance as to justify their consideration in greater detail.

The maximum rise in the pH value in all cultures occurred in those containing nitrates, whether carbon was supplied as oil, starch or sugar, a peculiarity noted also in the original agar cultures (see Tables I and II). Viewed in relation to the intolerance of the host plant to alkalinity in the rooting medium and to more than minimal amounts of nitrates in experimental cultures, this observation becomes significant (Rayner, 1922, 1925).

Calluna flourishes naturally in acid moorland soils of low pH and grows well in pure culture in solutions or in agar gels at pH 3.8 and lower. It is abundant also in heathy soils of reaction about pH 5.0 and has been observed growing well in heavy loam of pH 7.0 (Rayner, 1911). The species is notoriously sensitive to soil alkalinity brought about by a high lime content and there is experimental evidence for both *Calluna* and *Vaccinium* that the lethal effect is not due to calcium ions but depends upon a decreased hydrogen-ion concentration associated with the presence of calcium salts (Rayner, 1915). A similar reaction can be induced in *Vaccinium corymbosum* by increasing the alkalinity of the culture solution without alteration of the calcium content (Coville, 1911).

It is unlikely that this behaviour is a simple reaction to the hydrogen-ion concentration of the soil solution. The critical pH value for healthy growth of *Calluna* and other lime-shy species is probably determined by certain correlated soil factors and it is hoped that water culture experiments on *Calluna* and *Erica carnea* now in progress may yield more precise evidence with respect to this and so provide the data still required to explain not only the calcifuge habit but also the inconsistencies in this respect observed between

closely allied species or even between individual plants of the same species in different habitats.

Equally striking and significant is the reaction shown by *Calluna* seedlings in pure culture to the presence of nitrates in the substrate. Apart from its bearing on nitrogen fixation by the endophyte it is a remarkable fact that seedlings of *Calluna* in pure culture thrive better in nutrient media lacking nitrates or combined nitrogen in any form than in the same media containing nitrate (Rayner, 1922). Indeed, such nitrogen-free substrata appear to offer optimum conditions for the growth and development of "synthetic" seedlings in pure culture.

Recent observations by Pearsall and Ewing on nitrogen metabolism may provide a further explanation of the observed sensitiveness of *Calluna* to nitrate supply. These workers have shown that amino-acids tend to accumulate in plants provided with abundant nitrates. This influences "the metabolism in such a way that acid production is reduced with a consequent reduction in the hydrogen-ion concentration of the sap" (Pearsall and Ewing, 1929).

Consider now these observations in relation to one another: (i) *Calluna* forms healthy, active and typical mycorrhiza in acid soils very deficient in nitrates. (ii) In pure culture very favourable growth conditions are provided by the use of solutions free from nitrate (the development of mycorrhiza is not here involved since roots although infected by mycelium do not develop functional mycorrhiza in a sterilised inorganic substrate). (iii) In the rooting conditions provided by calcareous soil or soil solutions of high pH value associated with relatively large amounts of nitrate and other inorganic salts, young roots of *Calluna* show a remarkable investment of bacterial colonies pointing to the existence of a zone of increased alkalinity in the immediate neighbourhood of the root surface. Finally, (iv) In pure culture the endophyte is known to raise the pH of an acid substratum to pH 8.0 or higher when supplied with nitrates, a condition realised in the experimental cultures grown in calcareous soil or soil extract just described.

It is tempting to find a causal connection between these phenomena and to discover in them clues to a working hypothesis that will afford a sound and consistent explanation of the edaphic peculiarities of ericaceous species and of the complicated part played in them by the activities of their mycorrhizal endophytes. Such an hypothesis provides new and attractive material for critical observation and experiment. For example:—Is it possible to cultivate *Calluna* under

relatively alkaline soil conditions provided the nitrate and other salt content is as low as in the peat soils that form the natural habitat? Does the reaction of the mycorrhizal cells become more alkaline when the supply of nitrate is increased, and if so, is a similar effect produced by supplying combined nitrogen in other easily accessible forms? Is it possible to obtain evidence of differential behaviour in respect to nitrate supply on the part of endophytes of other ericaceous species that do not show the lime-shy habit, e.g. *Erica carnea*, *E. mediterranea* and certain *Rhododendrons*?

Answers must be found to these and similar questions before the facts relating to the metabolism of *Phoma radialis* now recorded can be linked directly with the edaphic behaviour of its host.

SUMMARY

1. A critical account is given of the occurrence of the endophyte of *Calluna vulgaris* within the fruit, and of the method used for extraction and isolation.

2. The decrease in vigour and loss of sporing capacity observed when the endophyte is sub-cultured continuously to the same nutrient are described.

3. The sudden appearance of a non-sporing "strain" or "saltation" which retains its characters when sub-cultured is recorded. The significance of the sectoring phenomena so produced in relation to observed irregularities in sporing habit is discussed.

4. The reaction of the fungus in pure culture to various nutrients is described, in particular to *Calluna*-oil, starch, sugars and nitrogen compounds. The behaviour on media lacking combined nitrogen is also considered.

5. Study of the endophyte in pure culture has provided considerable information as to the nature of its metabolic activities and thus afforded clues to its behaviour when growing symbiotically. This has made possible, in conjunction with other observations, the framing of a provisional hypothesis consistent with the edaphic peculiarities of the host.

The phenomena in question are discussed in some detail.

Acknowledgment is due to Professor J. H. Priestley for kindly supplying the *Calluna*-oil used in some of the cultures.

BEDFORD COLLEGE,
UNIVERSITY OF LONDON.
March, 1929.

Table I. Showing change of reaction in culture media brought about by growth of *Phoma radici* *Callunae*

Series	<i>Calluna-oil</i>			Starch		
	NO ₃	Pep- tone	Lacking combined N	NO ₃	Pep- tone	Lacking combined N
<i>pH</i> of control medium at end of experiment	4.8	5.2	5.0	5.4	5.9	4.8
1 <i>pH</i> of culture medium after 6 weeks	7.7	7.3	6.0	8.0	7.5	6.2
2 <i>pH</i> of culture medium after 6 weeks (another series)	7.3	7.2	6.3	7.7	7.4	6.4
3 <i>pH</i> of culture medium after 3 months	7.25	7.2	6.5	8.3	7.5	6.2
4* <i>pH</i> of culture medium after 5 months	Missing	6.7	6.8	7.7	6.9	6.2
Series	Saccharose			Dextrose		
	NO ₃	Pep- tone	Lacking combined N	NO ₃	Pep- tone	Lacking combined N
<i>pH</i> of control medium at end of experiment	5.2	5.2	5.3	5.3	5.3	5.3
1 <i>pH</i> of culture medium after 6 weeks	8.0	6.3	6.25	7.7	7.2	6.25
2 <i>pH</i> of culture medium after 6 weeks (another series)	7.7	7.3	6.85	7.4	7.2	6.9
3 <i>pH</i> of culture medium after 3 months	7.7	7.2	6.5	7.7	7.2	6.5
4* <i>pH</i> of culture medium after 5 months	7.7	6.85	6.9	7.3	6.95	6.8

Table II. Summarising analyses of cultures on liquid media

Culture	Dry weight (gm.)	pH	Starch	Dextrin	Sucrose	Glucose	Ammonia	Fatty acids	Carbonates	Nitrates
Starch-peptone	—	5.4	+	—	—	—	—	—	—	—
1 week	0.0056	5.4	+	+	—	—	V. slight	+	—	—
2 weeks	0.2114	7.5	—	+	—	—	+	+	—	—
4 "	0.1651	7.8	—	+	—	—	+	Slight	—	—
7 "	0.1526	8.0	—	+	—	—	+	"	—	—
Starch-nitrate	—	4.8	+	—	—	—	—	—	—	+
1 week	0.0014	4.8	+	+	—	—	V. slight	—	—	+
2 weeks	0.0755	6.9	—	+	—	—	Slight	—	—	+
4 "	0.1787	8.2	—	—	—	—	+	—	—	—
Starch alone	—	4.9	+	—	—	—	—	—	—	—
1 week	0.0011	3.8	+	+	—	—	—	+	—	—
2 weeks	0.0085	5.6	+	+	—	—	—	+	—	—
4 "	0.0196	5.0	+	+	—	—	—	Slight	—	—
Oil-peptone	—	5.3	—	—	—	—	—	—	—	—
2 weeks	0.0851	7.0	—	—	—	—	+	+	—	—
4 "	0.1869	7.8	—	—	—	—	+	Slight	—	—
7 "	0.1494	8.1	—	—	—	Slight	+	"	—	—
Glucose-peptone	—	5.3	—	—	—	+	—	—	—	—
1 week	0.0101	5.3	—	—	—	+	—	+	—	—
2 weeks	0.2488	7.0	—	—	—	Slight	+	+	—	—
4 "	0.1916	7.7	—	—	—	—	+	+	—	—
7 "	0.1772	8.1	—	—	—	—	+	+	—	—
Glucose-nitrate	—	4.9	—	—	—	+	No test	—	—	+
1 week	0.0038	4.9	—	—	—	+	—	"	—	+
2 weeks	0.1428	7.0	—	—	—	+	—	? Trace	—	+
4 "	0.2042	8.2	—	—	—	—	—	—	—	—
Glucose alone	—	4.7	—	—	—	+	—	—	—	—
1 week	0.0016	3.9	—	—	—	+	—	+	—	—
2 weeks	0.0123	5.3	—	—	—	+	—	+	—	—
4 "	0.0022	5.3	—	—	—	+	—	+	—	—

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EXPLANATION OF PLATE VI

Fig. 1. *Phoma radialis Callunae*. Three cultures of same age and identical history. (a) Sporing freely after 10 days' growth. (b) Sporing scantily after 10 days' growth. (c) Not sporing after 30 days' growth (or subsequently).

Figs 2, 3, 4. Sectoring colonies on various media.

Fig. 2. Glucose agar, 27 days. Fig. 3. Saccharose agar, 27 days.

Fig. 4. *Calluna*-oil agar, 14 days.

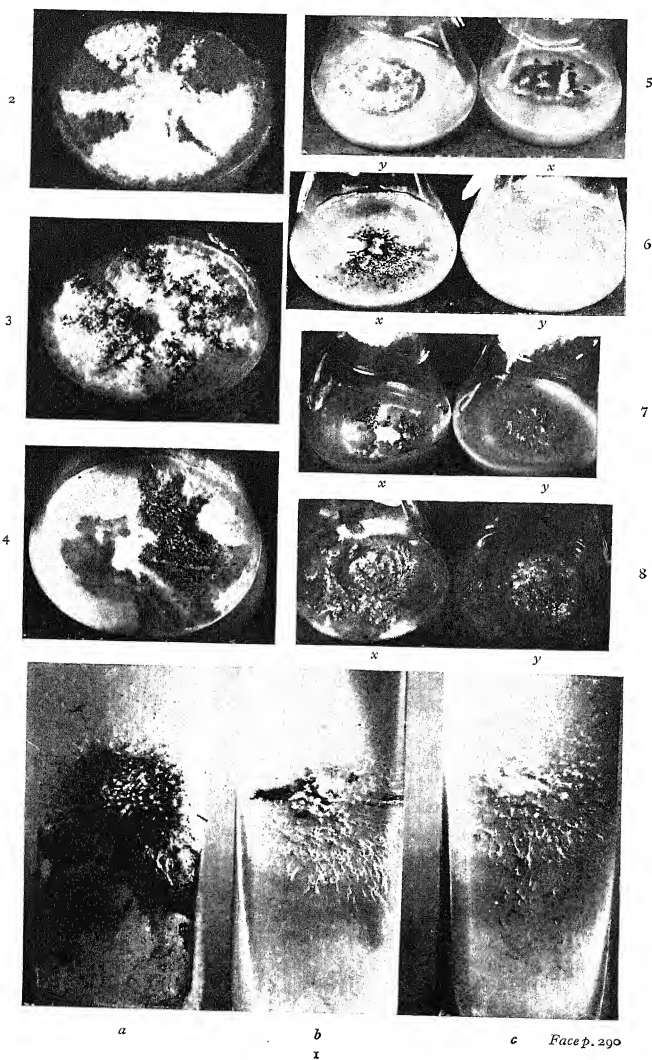
Figs. 5, 6, 7, 8. Sub-cultures of various generations from sporing area, (x), and non-sporing area, (y), of plate shown in Fig. 4.

Fig. 5. Saccharose agar: x, sporing strain, y, non-sporing strain.

Fig. 6. *Calluna*-oil agar: x, sporing strain, y, non-sporing strain.

Fig. 7. Saccharose agar: x, sporing strain, showing "secondary" sectoring, y, non-sporing strain.

Fig. 8. Potato agar: x, sporing, y, non-sporing strain, young (5 days) colonies antecedent to sporing showing differential vegetative growth.

RAYNER AND LLEWELLYN SMITH—*PHOMA RADICIS CALLUNAE*



PLANT ELECTRICITY¹

II. TOWARDS AN INTERPRETATION OF THE PHOTO-ELECTRIC CURRENTS OF LEAVES

By J. C. WALLER

(With 8 figures in the text)

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INTRODUCTION

IN continuation of a previous publication (Waller(3)), further experimental observations concerning the photo-electric response of leaves are here presented, together with deductions tending towards an interpretation of this mysterious phenomenon.

The photo-electric currents which are so marked a characteristic of chlorophyllous plant organs can be brought into evidence by means of a galvanometer in circuit with a leaf. Connection with the leaf is made through two unpolarisable electrodes, as described fully in the previous paper. One of these electrodes is in contact with a portion of the leaf (the "experimental" portion) which may at will be exposed to light or plunged in darkness. The other electrode is in contact with a portion of the leaf (the "control" portion) which is kept under relatively uniform conditions throughout the experiment. As the control we may utilise (1) a portion of the leaf shielded by black paper so that it is kept constantly in darkness; (2) a portion

¹ The first paper of this series was published in the *Annals of Botany*, 39, 516-538, 1925.

kept constantly illuminated; (3) an albino portion of a variegated leaf. All of these methods have in my experience yielded similar results, but the first method, which was that originally introduced by my father, the late Prof. Augustus D. Waller, in his pioneer researches upon this subject, is by far the most convenient, and has been used in nearly all the experiments of the present paper.

THE PHOTO-ELECTRIC CURRENT IN NATURE

If a leaf is set up as described, it will be found that any change in the intensity of light falling upon the experimental portion of the leaf will bring about an immediate change of potential in the leaf. Fig. 1 shows the countless alterations of potential in a leaf set up near a south window on a sunny day when small clouds were constantly passing across the sun.

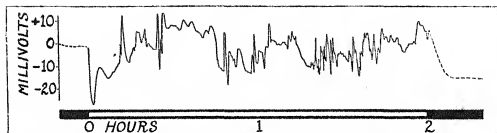


Fig. 1. Geranium (*Pelargonium zonale*): 2 hours' exposure to sunlight. The irregular changes of potential are caused through variations of illumination due to clouds passing across the sun. (Record 104.)

N.B. In this and other figures exposure to light is marked by a horizontal white bar below the record. Upward deflection indicates positivity of the exposed surface of the leaf, downward deflection indicates negativity (see also text).

This record suggests the myriad electrical fluctuations which are likely taking place in the leaves of a tree, with shadows of leaves continually flickering across each other. Such activity, though not perceptible to us, may be compared to the myriad points of light when the sun glitters on the sea.

ARTIFICIAL LIGHT

If a leaf is exposed to light of constant intensity, i.e. bright electric light, a series of fluctuations of potential occur which tend to subside as illumination is prolonged (see Fig. 2). When the light is cut off a similar series of fluctuations are again brought about, but commencing in the opposite direction from that on first illumination.

It should here be noted that in all my records upward deflection of the tracing indicates a positive change of potential in the experimental portion of the leaf, i.e. current within the leaf towards this portion and away from the control portion. Vice versa, downward deflection indicates a negative change of potential in the experimental portion, i.e. current within the leaf away from this portion and towards the control portion.

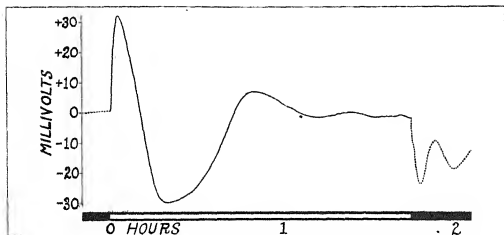


Fig. 2. Cabbage: $1\frac{3}{4}$ hours' exposure to electric light. (Record 112.)

ORIGIN OF THE CURRENTS

If, as appears unquestionable (Waller(3)), the photo-electric currents are intimately bound up with photosynthetic activity, why do these currents tend to subside during illumination and why do they again occur immediately the leaf is plunged in darkness?

It appears to me that this may most simply be accounted for by conceiving the presence of an acid as an incident or phase attendant alike upon photosynthetic and respiratory processes. This acid might be an intermediate product or else a by-product. Now oxidation may be defined as loss of electrons, reduction as gain of electrons (Stieglitz(2), p. 252). Thus oxidation of the acid would cause a negative change of potential: reduction a positive change. When oxidation and reduction equalise each other potential would be at zero. When the leaf is subjected to a change of illumination the equilibrium between oxidation and reduction would be upset, only to be re-established again under the uniform condition either of prolonged illumination or of prolonged darkness. Before, however, it is re-established in either case, there appears to be a see-saw between oxidation and reduction expressed as the gradually subsiding series of negative and positive electrical fluctuations.

The above hypothesis is in harmony with the following observational facts. 1. Under anaerobic conditions the positive phase—dependent upon reduction—tends to predominate over the negative phase—dependent upon oxidation (see “Effect of Nitrogen and of Hydrogen” below). 2. The positive phase is also greatly accentuated in a leaf which has been kept in darkness for a number of hours previous to experiment. This is explicable if such a leaf is regarded as being richer in acid and poorer in free oxygen than a leaf which has been for some hours in the light (see below under “‘Darkness’ leaves and ‘Light’ leaves”). 3. Atmospheric carbon dioxide also favours the positive electrical phase. This is in harmony with the fact that carbon dioxide acts as an acid (see observations below).

Before entering upon these details the types of electrical reaction to be met with in various kinds of leaves will be referred to.

STUDY OF VARIOUS LEAVES

The great majority of leaves on first illumination give a negative deflection, and on cutting off the light a deflection in the opposite (positive) direction. An example is in Fig. 3 *a* which shows a record of *Pelargonium zonale*, the garden geranium. Large numbers of

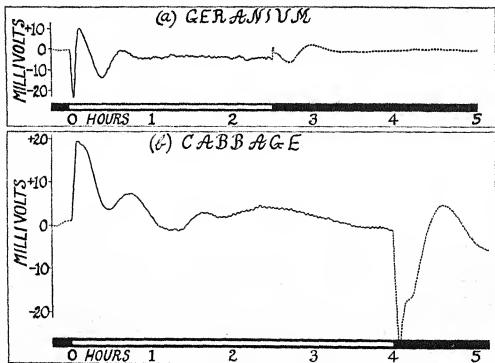


Fig. 3. (a) Geranium: $2\frac{1}{2}$ hours' illumination. (Record 502 e.)
(b) Cabbage: 4 hours' illumination. (Record 130.)

The initial effect of light in Geranium is negative, the initial effect of darkness positive. In Cabbage these effects are reversed.

leaves of this plant tested during the last few years have never failed to act in this manner. During May and June 1924, with the help of a friend, about 50 different species of leaves were tested and they all acted like *Geranium*. On the other hand, young pale green Cabbage leaves are different and have always given a positive effect on first illumination and a negative deflection on cutting off the light (see Figs. 2 and 3 *b*). Leaves of trees (9 species), of woody plants (7 species), of succulents (2 species), of Monocotyledons (5 species) and of plants of shady habitat (3 species) have been found to give the most prolonged negative deflection on first illumination (e.g. Rose, Fig. 4), while in herbs whose leaves form abundance of starch, belonging to the families Cruciferae (6 species), Papilionaceae (3 species) and Solanaceae (2 species), the first negative deflection is less prolonged (e.g. Turnip, Fig. 4).

Leaves which have been found to act like Cabbage are the mature leaves of Golden Privet (Fig. 4) and etiolated pale green parts of plants (e.g. Broad Bean, Fig. 7 *a*). It seems possible that this latter action is associated with a greater reducibility due to an anaerobic condition within the tissues.

The effect of various conditions upon the response will now be described.

"DARKNESS" LEAVES AND "LIGHT" LEAVES

If a leaf is kept in darkness (say overnight or during a whole day) and then tested, the positive phase of its response has been found to be markedly augmented. Its response becomes like that of an etiolated leaf, but the full green colour remains. A leaf in this condition I therefore call a "darkness" leaf. If on the other hand the same leaf is left exposed to sunlight for some hours and then again set up for experiment and tested, the negative phase again comes into evidence. A leaf in this condition I call a "light" leaf. *Nasturtium* (*Tropaeolum majus*) has proved a good leaf for showing this phenomenon (see sample records in Fig. 5). Similar phenomena were shown in the case of *Geranium* in two series of observations continued during a number of consecutive days.

If a "darkness" leaf be regarded as in a more anaerobic condition than a "light" leaf, i.e. richer in acid and poorer in available oxygen, the dominance of the positive (reduction) phase in the response of such leaves is explicable. Any slight dislocation in the chain of chemical reactions whereby carbohydrate is oxidised to carbon dioxide and water might result in such an accumulation of acid.

The leaves of fleshy or succulent plants are known to form a store of acid in this manner and it seems not impossible that some similar tendency, though of course not so marked, may exist in the leaves of ordinary plants.

In this connection also it is interesting to note that Dutrochet (11, p. 559 *et seq.*) regarded plants which had been in darkness and also etiolated plants as in a state of asphyxia or lack of oxygen.

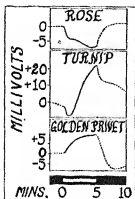


Fig. 4

Fig. 4. Different kinds of leaves. Negative response in Rose: negative rapidly giving place to positive in Turnip: positive in Golden Privet. (Records 236, 264, 218.)

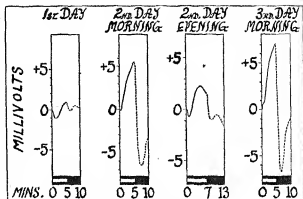


Fig. 5

Fig. 5. "Darkness" leaves and "Light" leaves. Nasturtium (*Tropaeolum majus*). The 1st and 3rd records were taken after the plant had been a number of hours in daylight, the 2nd and 4th after a number of hours in darkness. Same leaf used throughout. (Records 45, 46, 47, 48.)

EFFECT OF NITROGEN AND OF HYDROGEN

A number of experiments were performed with detached leaves set up in a small glass chamber through which a stream of air or other gas could be passed, suitable precautions being taken to keep the leaf supplied with moisture.

The most clear-cut results were obtained by testing with short periods of illumination at intervals. To save labour an automatic device was installed by which the leaf was exposed to periods of illumination of 3 minutes, each illumination being followed by a 17-minute period of darkness. The records were usually of 5 hours' duration, thus permitting of 15 tests during each record.

Fig. 6 shows results with Green Privet, a leaf which gives a negative deflection on first illumination: also with Golden Privet, a leaf which gives a positive deflection on first illumination. In the first case the negative responses in air are seen to be replaced by positive responses in nitrogen. In the second case the positive

responses in air are seen to be augmented in nitrogen. Thus in both these types of leaf the same tendency is observable in nitrogen, viz. an augmentation of the positive phase. This may be attributed to a diminution of the oxidatory negative phase through lack of oxygen.

Records confirmatory of the above were obtained also with *Nasturtium*, *Geranium*, *Dahlia* and *Cabbage*.

In the record of *Green Privet* it will be noticed that the response diminishes as treatment with nitrogen continues. A similar diminution was observed in *Geranium* but was more pronounced, culminating in complete extinction of the response.

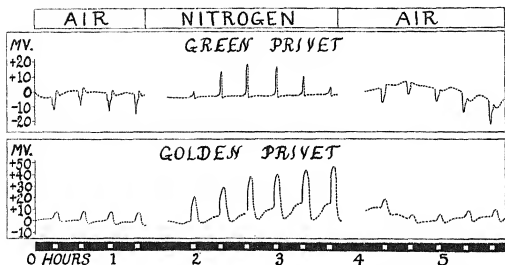


Fig. 6. Effect of nitrogen (absence of oxygen). The negative response of *Green Privet* becomes positive while the positive response of *Golden Privet* becomes still more positive. 3-minute illuminations with 17-minute periods of darkness. (Records 427, 470.)

Hydrogen acts in the same way as nitrogen but more rapidly, a fact explicable by the more rapid diffusibility of hydrogen. Thus with *Green Privet*, *Golden Privet*, *Geranium* and *Hyacinth* very rapid abolition of the response took place during the passage of a current of hydrogen. In the case of the fern *Pteris cretica* and of the French Bean, both of which gave negative deflections in air, positive deflections were shown in hydrogen followed by more or less complete abolition. The records with *Dahlia* and *Broad Bean* (negative deflections in air) and with *Cabbage* (positive deflections in air) are somewhat irregular, but in all cases there is a marked diminution of the response in hydrogen followed by recovery in air.

EFFECT OF CARBON DIOXIDE (CO_2)

Attention is drawn to the two records in Fig. 7. The upper record is with a young pale green leaf of Broad Bean, which gives an initial positive deflection on illumination. The periods of illumination are one hour each. It will be observed that in CO_2 -free air the negative fluctuations are strongly marked, whereas in normal air they are almost damped out by the predominant positive effect. The lower

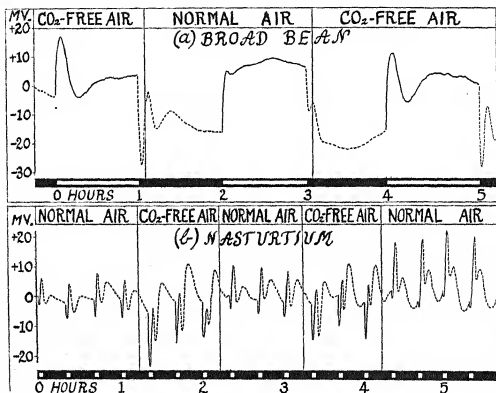


Fig. 7. Effect of atmospheric CO_2 .

(a) Broad Bean: 1-hour illuminations. In normal air the negative phase of the response is damped out. (Record 318.)

(b) Nasturtium: 3-minute illuminations with 17-minute periods of darkness. Here also the negative phase comes more into evidence in CO_2 -free air, while in normal air the positive phase is enhanced. (Record 487.)

record is with a leaf (Nasturtium) which gives an initial negative deflection on illumination. Three-minute periods of illumination at intervals are here employed. It will be noticed that in normal air the initial negative deflection rapidly gives way to the positive phase, whereas in CO_2 -free air the initial negative deflection is augmented.

Thus in both the above leaves the same tendency is traceable through the records, viz. an enhancement of the positive phase in the presence of atmospheric carbon dioxide.

It should be added that quite a number of indecisive results with different kinds of leaves were obtained before this action of atmospheric CO_2 was successfully demonstrated. It was in mid-winter with Broad Beans, forced in a greenhouse, that six decisive records were obtained. Three records taken a few weeks later proved indecisive. The record of *Nasturtium* was made in July.

The fact that the presence or absence of atmospheric CO_2 is so often without effect is not surprising when one considers the part played by internal conditions upon the photo-electric response, as shown in the section on "darkness" leaves and "light" leaves. Such internal action, if strong, would damp out the action due to the small quantity of CO_2 in the air.

Results in harmony with the above have been obtained by adding CO_2 above that normally present in the air. A record of *Dahlia* shows augmentation of the negative phase in CO_2 -free air as compared with normal air, while in an atmosphere containing 3 per cent. CO_2 the positive phase is greatly augmented. Eight records with Cabbage show augmentation of the positive effect in the presence of extra CO_2 , while one record of *Geranium* and one of *Pteris cretica* show clearly a diminution of the negative phase.

When a leaf is in pure CO_2 the response entirely disappears, but reappears again in air.

THE PHOTO-ELECTRIC RESPONSE COMPARED TO THE ACTION OF A WEATHERVANE

The photo-electric response may be represented by means of the following similitude.

Picture a weathervane in a wind blowing steadily from one direction: the weathervane will remain at rest pointing in this direction. If the wind changes suddenly and blows from the opposite direction, the weathervane will instantly veer round till it points in the opposite direction; but before taking up its new position it will be seen to overshoot the mark and to undergo a series of gradually subsiding oscillations like a pendulum, until it comes to rest.

Now let the chemical change of materials in photosynthesis be represented by a north wind and let respiration, the reverse process, be represented by a south wind. Let the hypothetical acid, to which I ascribe the photo-electric response, be represented by the weathervane.

The electrical fluctuations which occur in a leaf after a change from light to darkness or from darkness to light are now seen closely

to resemble the oscillations undergone by the weathervane before it comes to rest after a change in direction of the wind (see Fig. 2). The oscillations of the vane may well be taken to represent the see-saw of oxidation and reduction in the acid medium which appears to occur every time photosynthesis is replaced by respiration or respiration by photosynthesis, and the photo-electric response itself is merely the expression of this see-saw which continues until equilibrium of oxidation and reduction is again established.

This figment is remarkably apt, as will be acknowledged after the following further considerations. If an improvised weathercock is placed in the wind from an electric fan in the laboratory, it will be noticed that the vane never comes absolutely to rest but remains constantly in a state of tremor. A similar phenomenon is often discernible in records of the photo-electric response (see especially Fig. 3 *b*), for here it is seen that as the first fluctuations of potential subside during illumination, a series of small fluctuations or tremors make their appearance, as if two processes (oxidation and reduction) were almost but never quite in equilibrium with each other. Furthermore, we may compare rate of photosynthesis to wind velocity; for observation of a weathervane will show that the stronger the wind, the more rapidly the vane comes to rest in its new position after a change in the direction of the wind. Its oscillations are of greater amplitude and of shorter duration than in a wind of less velocity. A similar relation holds between rate of carbon-assimilation and photo-electric fluctuations of potential, as will be shown in the following section.

COMPARATIVE STUDY OF THE RATE OF CARBON-ASSIMILATION

In order to make concurrent records of the photo-electric response and the activity of photosynthesis, a small glass chamber was constructed provided with two tubes for the electrodes and an outlet communicating with a Katharometer made by the Cambridge Instrument Company. This instrument enables one to make a continuous record of the concentration of CO_2 present in the atmosphere in the glass chamber, and with certain precautions the rate of respiration and also of photosynthesis may be measured (Waller⁽⁴⁾). By this arrangement records were obtained showing upon the same piece of paper the photo-electric response and also the rate of CO_2 absorption or emission by a single leaf.

Fig. 8 shows the photo-electric responses given by a leaf of *Nasturtium* at light intensities of 1, 2, 4, 8 units respectively, unit

intensity being the light given by a 1000 candle-power "Pointolite" lamp at a distance of 60 cm. from the leaf. Inspection of the four tracings will show that with increasing light intensity the fluctuations become of greater amplitude and of diminishing period. Rates of carbon-assimilation in milligrams CO_2 absorbed per 100 sq. cm. leaf surface per hour were respectively 9.3, 20.1, 30, 42.3.

Thus the photo-electric fluctuations vary with the rate of carbon-assimilation in a similar manner as the oscillations of a weathervane vary with the velocity of the wind.

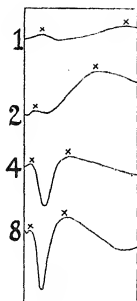


Fig. 8. Response of *Nasturtium* at different light intensities—represented by numbers on the left: 22-minute illuminations. (Record 451.)

SUMMARY

1. Three methods of observing the photo-electric currents of leaves are enunciated.

2. Indication is given of the myriad fluctuations of potential which are likely occurring in leaves under natural illumination.

3. The following description of the photo-electric current in terms of physical chemistry is given. The positive phase of the current is attributable to the reduction of an acid, the negative phase to its oxidation. The current would thus be the result of a lack of equilibrium between oxidation and reduction. This equilibrium appears to be upset by any *change* of illumination, but is re-established under the condition either of prolonged uniform illumination or of darkness.

4. The above hypothesis is borne out by the fact that under anaerobic conditions the positive (reduction) phase of the response is enhanced.

5. Most leaves show an initial negative phase on illumination, but etiolated leaves, leaves poor in chlorophyll and leaves which have been kept for a number of hours in darkness show an initial positive phase.

6. The electrical fluctuations brought about in a leaf by a change of illumination resemble the oscillations of a weathervane after a change in direction of the wind, and they appear to me to be of no more importance to photosynthesis than is a weathervane to the wind.

I desire to express my profound thanks to Prof. J. S. Macdonald, Professor of Physiology in the University of Liverpool, for his hospitality and kindness in making possible the carrying out of these researches.

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EVOLUTION OF THE TERTIARY FLORA IN ASIA

BY PROF. A. N. KRYSHTOFOVICH

(Leningrad)

ASIA is a region which has furnished abundant and well-preserved remains of Tertiary plants, which have been described by the best students of palaeobotany, and it is also the region which has preserved in the completest form those communities of plants which inhabited Europe in the earlier and later Tertiary epochs. In the tropical parts of Asia, especially in the Malayan region, are best preserved the plants which flourished in Europe during the Eocene and part of the Oligocene epoch; whereas South-Western China and Japan have preserved the type of vegetation which inhabited Europe and the middle zone of Asia (Western Siberia, Altai, etc.) during the later Tertiary, indeed until the oncoming glaciation destroyed nearly all types of plant life. As is well known, not only did the glaciation put an end to the gradual development of the flora, but mountain chains, already in existence, hindered the free migration of vegetation and prevented the return movement to places where conditions were quite favourable for the existence of the former inhabitants after the ice cover had disappeared.

It seems rather strange that the abundance of fossil floras known from Northern Asia failed to suggest of themselves any leading idea regarding the evolution and migration of floras on this great continent. The chief reasons for this failure were that several important floras were confused with others of a different age, and that some were not determined quite correctly. Furthermore, ideas now discarded played a leading part in the discussions. From such causes it followed that certain alien elements were introduced as forming part of floras to which they did not belong, and there resulted a sameness of outlook which prevented further progress and caused some students to reject various palaeobotanical conclusions as unreliable. These results arose chiefly from the inaccuracy of geologists when making their collections. For instance, from 1878 until 1918(1) *Nilssonia serotina* was regarded as a Tertiary plant which occurred in Sakhalin so late as the Miocene. Hence even such an authority as A. G. Nathorst was misled into believing that possibly somewhere

in the wilderness of China a living *Nilssonia* might be found. But the supposed Miocene proved not to be later than Senonian (Cretaceous). Again, the famous Simonova flora from middle Siberia, which is so often cited, was described by O. Heer⁽²⁾ as Tertiary in spite of the fact that its *Eucalyptus* was in discordance with the evidence derived from all the neighbouring Tertiary floras. The Simonova flora is purely Cretaceous. Again, whilst the Simonova collection is unmixed Cretaceous, that from Sakhalin was mixed, containing both Cretaceous and Tertiary specimens introduced by careless collecting. Another flora from the mouth of the Bureya river, a tributary of the Amur, described by Heer as Miocene⁽³⁾ is doubtless the equivalent of the Laramie flora, i.e. Upper Cretaceous; probably it is exactly the age of the Danian, a conclusion which is confirmed by the discovery in the strata of dinosaurs⁽⁴⁾.

Now, at last, the rehabilitation of certain among the Asiatic floras, and the more recent study of others, have begun to show the Tertiary flora of Northern Asia in its true character. It is this fact, with some considerations based on my personal studies of the fossil floras of Eurasia from the Polish border to Sakhalin, Japan, and the Philippine Islands, which now enables me to try to outline the former botanical provinces of Asia, and to trace the principal movements of these floras and their evolution, as evidenced by fossil remains.

But, since even at the present time the real age of most Asiatic floras is often mistaken in Europe—for instance, Köppen and Wegener⁽⁵⁾ lately cited them not quite correctly—I shall begin by stating the ages of the chief of these floras (from west to east) as they are regarded by myself.

Northern Urals (Lozva River)	Lower Oligocene—Upper Eocene
Aral Sea, Tomsk, etc. (several localities)	Upper Oligocene—Lower Miocene
Irtys River, near Tara	Miocene
Bukhtorma in Altai Mountains	Miocene (Upper Oligocene?)
Simonova on the Chulym River	Upper Cretaceous
Baikal Lake	Miocene (Upper Oligocene?)
New Siberia Islands	Eocene?
Anadyr in Chukchaland	Eocene (Fort Union?)
Tas-takh Lake in Yakutia	"
Amur-Zagayan (mouth of Bureya)	Danian (uppermost Senonian?)
Kalgan, Mongolia	Oligocene
Fushun, Manchuria	"
Sikhota-alin (several localities)	"
Mgach (in part) and some other localities, Sakhalin	Cenomanian to Senonian
Dui and other localities (Mgach in part)	Lower Oligocene, may be partly younger and partly also older
Kamchatka, Commodore Islands	Oligocene
Hokkaido (several localities)	Eocene—Miocene

Honshu and Kyu-shu, Japan

Eocene—Pliocene, partly Quaternary

Alaska (*Trapa borealis*-flora)

Oligocene

Philippine Islands (Vigo and Malumbang)

Lower Miocene and Pliocene

Besides these, other data have been published by Prof. V. N. Sukachev⁽⁶⁾ and myself⁽⁷⁾ on the Quaternary flora of Siberia, and in 1914 I found on the Amur impressions of *Ginkgo* and *Zelkova* which also seem to me to be Quaternary.

The work I had already done on the floras mentioned gave rise to ideas which I was prepared to develop when I got for study a collection of fossil plants from the Lozva river in the Urals in lat. 61° 10', collected as long ago as 1884 by the famous Prof. Eugraph Fedorov; from this I was able to establish the following species: *Pecopteris Torellii* Heer, *Acrostichum* sp., *Potamogeton uralense* n.sp., *Sequoia Sternbergii* Goepf., *S. Langsdorffii* (Brongn.), *Ficus uralica* n.sp., *Populus Richardsonii* Hr., *Magnolia Inglefieldii* Hr., *Ilex longifolia* Hr., *MacClintockia trinervis* Hr. and *M. Lyellii* Hr., besides some fragments of other types.

The presence of so peculiar a plant as *MacClintockia*, as well as the specific composition of the flora, has led me to recognise in it an exact equivalent of the Tertiary flora of Greenland formerly described by Heer.

The sharp distinction of this flora, on the one hand, from the early Tertiary floras of the Ukraine and of West Europe, and on the other from the floras which inhabited Siberia and North Turkestan, has permitted me to express certain views concerning this problem.

The age of the Ural flora is not very easy to decide, for no other fossils were found in the locality, which is isolated amidst Palaeozoic country, while strata containing Eocene and Oligocene marine fauna are met with only at a considerable distance. But we may consider in this connection the Greenland Tertiary flora. This is now considered to belong to two successive stages: an older pre-basaltic stage with a flora containing *MacClintockia* but lacking most of those Amentiflorae which become so dominant in the later or basaltic stage. As the Ural flora corresponds with the pre-basaltic flora, its age should be considered as being Middle or Lower Oligocene, although even Upper Eocene is not excluded. Meanwhile it is quite possible that the same flora in Greenland itself is somewhat older, when account is taken of its slightly more northern situation, and at the same time the view be accepted that climatic zones were

already more or less in existence during the Tertiary period. Our knowledge of the marine Tertiary strata as it is known along the Eastern Urals does not contradict this statement.

The biological aspect of the plant association known from the Lozva river teaches us that from that locality any traces of real tropical affinities are quite lacking; which fact is in full accord with the statement made above concerning Greenland. This conclusion differs somewhat from the earlier opinion of O. Heer who, owing to mistaken identifications, attributed to certain Greenland plant remains properties which they do not possess. For instance, we now recognise the entire absence of palms from Greenland during the Tertiary period, because the specimens referred to the so-called *Flabellaria Johnstrupii* Heer really do not represent remains of plants at all. The plants of the Lozva I regard as temperate forest types mostly having deciduous foliage, and resembling the types at present growing in Eastern Asia and the Eastern States of North America. This Ural flora has no features in common with the Paleogene flora of Western Europe and of Ukraine, which show many characters indicating its tropical or subtropical nature; for instance, *Oreodaphne*, *Cinnamomum*, *Nipa*, *Sabal*, Myrtaceae in the Ukraine and a much greater number and variety of forms in West Europe. This difference between the floras under consideration must be attributed chiefly to the difference in their geographical positions, and not to their age, which is only accountable for a slight difference, since the Ural flora, together with its temperate aspect, still preserves such archaic features as *MacClintockia* and *Populus Richardsonii* which stand in close connection with Cretaceous types. The same mixed character is typical of the flora of West Greenland.

Examining the climatic conditions in which such a flora could develop, a flora distinct even from that which flourished, possibly a little later, in the region of Tomsk and the Aral Sea, I have come to the conclusion that during the Oligocene the Ural flora would postulate a mean annual temperature of about 10° C. The climate, and especially the degree of warmth required by Tertiary floras, is often somewhat exaggerated, especially when into these floras are introduced, erroneously, elements which are alien, and so produce a southern impression which the flora by no means possesses. At the present time *Magnolia* reaches even the Southern Kurile Islands, the palm *Trachycarpus* grows in Japan as far north as Sendai, certain plants of definite Tertiary extraction extend even farther north, to Sakhalin, and the maiden-hair tree (*Ginkgo*) is cultivated as far north as

Viborg in Finland¹; whilst on the other hand in Hokkaido minima of -41.0° C. occur. All these facts show that we must not represent the temperate Tertiary flora as growing in an especially warm climate, but that the essential character was rather the absence of rapid and great changes of temperature, a character found in the present Atlantic climate. In contrast to this the essential features of the Eocene and Oligocene floras of the Ukraine and Russia in Europe (and still more of West Europe) show that the mean annual temperature on the 50° of north latitude was not less than $18-20^{\circ}$ C. In these floras *Nipa* and *Sabal* palms occur, together with characteristic tropical and subtropical (extra-temperate) types. The conditions observed in the Ukraine were identical with those observed in England, Belgium, France and Germany, perhaps a little more temperate. Unfortunately the richest Eocene and Oligocene floras of England have only been partially studied.

Turning now to the Tertiary Siberian floras we are not able to find in them any remains that would permit us to suggest the existence anywhere in this country of strictly tropical or subtropical conditions during the Tertiary period. On the contrary all the known facts concerning the territory which stretches from Turgai (8-10) and Tomsk city (11) on the west to Vladivostok, Corea and Sakhalin on the east, demonstrate the former distribution there of a flora composed mostly of temperate types such as *Fagus*, *Ulmus*, *Alnus*, *Betula*, *Corylus*, *Populus*, *Juglans*, *Comptonia* and *Trapa*, and almost devoid of evergreen elements showing a southern character; or the southern plants, if present, are rare and doubtful. There are no palms, nor are there cinnamons or figs of a tropical type such as are so conspicuous in the older Tertiary flora of Europe. Some elements, such as *Ficus tiliacifolia* or *Buettneria aequalifolia* which are typical of these floras, seem to be of very doubtful generic relationship, their true nature being still questionable. It is possible that in them we see relics of the primaeval flora which has lingered on from such early times as the Upper Cretaceous.

In proceeding farther east we find the same monotonous Tertiary flora passing across the Pacific into Alaska, and found in an association exactly corresponding with the Dui Series in Sakhalin, without anywhere taking on a more southern aspect. Recently B. Kubart⁽¹²⁾ has rightly pointed out some erroneous identifications which have led to quite unreasonable conclusions. However, whilst

¹ I have read this statement but cannot prove it now by citing the source of the information.

in Siberia proper any traces of a former much warmer climate and an associated flora are lacking, some traces of these phenomena are found in Turkestan and in Japan. Indeed, near Kushka, Turkestan, on the Er-oilan-duz lake, in the Oligocene strata, *Dryandra Schrankii* Brongn. and *Celastrophyllum turcomanicum* Krysht. were found—on the whole a flora of a kind not found elsewhere on the continent of Asia. It is probable that the Er-oilan habitat is the most eastern of the subtropical floras, having emerged from Europe by way of the Mediterranean region as far as the Himalayas. Moreover, it seems that this flora shows characters indicating more arid conditions than those which existed at the same time farther north. This fact seems to show evidence of a desiccation which had already begun in those regions early in the Tertiary. Other traces of a flora of more southern type in the extra-tropical zone of Asia were discovered in Kyūshū Island, where, in the Eocene beds of the Takashima Group, I have determined (13) a palm *Sabal nipponica* Krysht. and the ferns *Lastraea japonica* Krysht. and *Acrostichum hesperium* Newb.; the latter, however, by no means seem to be indicators of purely tropical conditions. In the Miike Colliery on the same Kyūshū Island, and in the same strata, are even found impressions of *Cycas* (14). All these data stand in full accord with facts of zoo-palaentology; for instance, numerous nummulites are found in the Lower Tertiary strata of Kyūshū, but are lacking in Hokkaido, where the Lower Tertiary flora, although still containing a *Sabal*, preserves a more temperate aspect.

Traces of somewhat more luxuriant conditions of growth than those found in the heart of the Asiatic Continent, but at the same time showing more arid conditions, are visible on the present margin of the continent in Ussuriland (15) and Corea (16), where, however, no traces of palms have so far been found: all the known plant associations consist chiefly of *Corylus*, *Castanea*, *Quercus*, *Fagus*, *Betula*, *Juglans*, *Alnus* and other common Arcto-Tertiary types, to which the nearly ubiquitous *Trapa* nuts and peculiar leaves of *Comptonia* add a very characteristic aspect. Even if remains of cinnamons were to be found there eventually they could not change very much the general character of this temperate flora, which is hardly warmer than that which at present so successfully withstands the low annual temperature of the country and its severe winter cold in Ussuri, Amur and North Japan. The rich composition of the flora of the Far East, even under severe climatic conditions, finds its explanation exclusively in a uniform quiet development under

climatic conditions gradually deteriorating, although possibly with some fluctuations. The plant world of the country has remained unmolested since time immemorial, not suffering such destructive phenomena as the formation of mountain chains prohibitive to plant migration, or glaciation such as that of Europe.

After examining the different Tertiary floras of East Europe and Asia I must express a belief that their different specific composition and morphological aspect cannot be explained solely by differences in their age, but must be attributed in great measure to their belonging to different floristic provinces.

The principal floristic boundary line should be traced in a north-west direction as separating the temperate flora of Arcto-Tertiary composition on the north-east from the evergreen tropical or subtropical flora of Europe including the Ukraine. I give to the latter region the name of the *Poltava Region* from the name of the Poltava sandstone or the Poltava stage. In this were found chiefly plant impressions characterising the evergreen older Tertiary vegetation of the Ukraine and South Russia with *Sabal*, *Oreodaphne*, *Cinnamomum*, etc.

In the Tertiary temperate region I separate first the *Greenland Province* embracing the Arctic territory, which was possibly a more homogeneous land mass than it is now, the northern Urals, and probably the adjacent parts of Europe and Asia. The flora of this province is well characterised by the fossil plants discovered on the Lozva, and by the flora of the pre-basaltic beds of Greenland. Among them *MacClintockia*, *Populus arctica* and *P. Richardsonii* are the most typical but, as a rule, the Amentiflorae such as *Alnus*, *Betula*, etc. are still scarcely developed. The vegetation of this province early in the Tertiary period consisted of temperate deciduous trees, as yet giving no indication of strict Asiatic or American relationship, but associated with genera which now extend far north such as *Magnolia*, certainly of early Tertiary origin and allied to types of the Upper Cretaceous flora.

The whole vast territory embracing the middle zone of Siberia, Northern Turkestan, Manchuria, Corea, Sakhalin, and the northern part of Japan, as well as, in America, Alaska and the adjacent territory, was in the first half of the Tertiary period, at least as late as the Lower Miocene, under the domination of a tediously monotonous summer-green forest flora. In this flora the Amentiflorae played an important part and *Trapa borealis* together with *Comptonia* (of the type *C. acutiloba* Heer, *C. japonica* Nath., *C. Naumannii*

Nath.) contributed easily recognisable elements. This province I term the *Turgai Province* from the name of a territory in which was discovered the flora first described by O. Heer from a find of H. Abich. Of course this huge territory is not quite uniform in its flora and could certainly be divided into several sub-provinces, but that must be done later after an exact study has been made of the composition and biological character of the floras concerned, as well as of their geological succession. The origin of this flora may probably be connected genetically with the basaltic flora of Greenland, which is best preserved at Haseninsel.

Besides this I think it possible to outline a separate *North Siberian Province* the flora of which, being possibly still older (Fort Union), is characterised by oval and circular leaves of an enormous size, which are certainly caducous. One may instance *Populus Richardsonii* of the type found in the New Siberian Islands(17), or in Anadyr(18). This flora still shows but a very distant relationship to the existing floras of either Eastern Asia or America, alliance with which is so remarkable in the later floras of Asia and Europe (Upper Oligocene to Late Pliocene). This North Siberian flora is remarkable for its *Nordenskiöldia borealis* Heer, gigantic leaves of *Populus* and *Pterospermities*, of which the later *Ficus tiliacifolia* is surely a morphological successor. The appearance of this flora, as well as its composition, seems to resemble closely the American Fort Union flora and the older Greenland flora and yet it is closely allied to the youngest Cretaceous flora of the Laramie type, namely that of the Tsagayan beds found on the Amur and Bureya(2), of which it is certainly a near descendant. Examples of such floras are those of the New Siberian Islands(17), of Anadyr(18) and of the Tas-Takh-Lake in Yakutia (not published); but all need further examination.

My principal aim is to outline the evolution of the flora of North Asia as shown exclusively by the fossil plants, but I will add a few words concerning the southern part of the continent to show the mutual relations between the two.

The vegetation of the southern part of Asia including the Sunda Archipelago has passed through an entirely different history from that of the changing flora of Europe or of the more constant flora of Northern Asia. In the South the flora has developed quite unmolested ever since its first descent from its Cretaceous ancestors, except in response to some slight changes in temperature and humidity which have taken place during this period. This is proved by fossil documents found in Borneo, Sumatra, Java, the Philippines

and other tropical countries, the Tertiary flora of which is, unfortunately, still but little known.

The temperate types of the Northern as well as of the Southern Hemisphere now find their refuge in these tropical regions in the high mountain zone, mostly above 1000 m. They penetrated into the tropics during a period of temperature depression (Pluvial period?) along the uprising mountain ranges, events which took place comparatively late. The lowland vegetation, on the other hand, has existed in these regions, practically unchanged, at least since the beginning of the Miocene period.

The distribution of botanical provinces during the Tertiary period as suggested above, the much warmer climate of Europe, and in contrast to it the slight change of climate (increased continentality) in North Asia, I am inclined to attribute to the migration of the North Pole to the northern part of the Pacific, with the corresponding changes in latitude of the countries under consideration; although in some degree these and other changes might be the result of displacement of either the whole, or parts, of the continental masses.

From such an explanation it would appear that, owing to the past history of Europe and Asia, respectively, it would be more natural that tropical relicts should be found in Europe than in the temperate parts of Asia, even than in its eastern part (other conditions exist in Turkestan where such relicts are really found in the present flora). But because in Middle and Northern Europe glaciation entirely destroyed the former vegetation, whilst in Southern Europe, in the Mediterranean region, the flora was changed by progressive desiccation, these relicts seem to occur, not in their original biological form, which was quite unfitted to survive the severe condition of the past and present, but under some disguise, for instance, in the form of mountain plants, as *Haberlea* and *Ramondia*, belonging to the Gesneraceae, or as xerophytic plants derived from the tropical types of the older Tertiary.

The necessary material is still scarce, but is rapidly growing, and the great continent of Asia seems to be destined eventually to disclose to us the life history of the present flora from its beginning, for in some parts of this continent the plants seem to have grown without interruption since they started to develop from their primaeval Cretaceous prototypes.

The assembling of conclusions derived from the study of different groups of plants and animals, both fossil and living, would certainly elucidate this problem more completely, but in the present paper I

have considered exclusively palaeobotanical facts, except when a few others are mentioned merely as illustrations of statements otherwise resting on a purely botanical basis.

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INFLUENCE OF AGE ON THE TEMPERATURE COEFFICIENT OF THE RESPIRATION RATE IN LEAVES OF *SCOLOPENDRIUM SCOLOPEN- DRIUM* KARST.

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It is a well established fact that the rate of plant respiration is markedly influenced by temperature and also the effect of age on the intensity of plant metabolism has been studied by many investigators. There is however little if any knowledge about the *simultaneous* action of both age and temperature on plant respiration. One of us (Bělehrádek⁽⁵⁾) has collected and analysed some earlier data from the zoological and physiological literature and has shown that the action of temperature on the rate of various biological processes is often different in individuals of the same species, but of different age. Thus the question seems justified, whether or not the action of temperature upon the rate of physiological phenomena in plants varies with age as it does in animals.

For this purpose we used leaves of *Scolopendrium scolopendrium* Karst., collected in April 1926 along a road from Brent to Brent Hill, near Plymouth. The leaves were kept on and covered with wet filter paper. They were classified into three groups:

- (i) very young leaves, still spirally coiled;
- (ii) fully formed young leaves, with the first signs of future soral ribs;
- (iii) old, deep green leaves of the last season, with the remains of last year's sporangia.

The oxygen intake of leaves of different groups was measured during the next three days after collecting by means of a small Barcroft gas analysis apparatus, previously calibrated. When putting leaves into the flask, care was taken not to injure them. Some of the readings were made with leaves freshly cut. It was ascertained from controls that cutting did not cause any difference in the oxygen intake. Nor did a difference of one or two days in the time which elapsed between collection and experiment have any marked effect on the respiratory metabolism.

The apparatus was immersed in a tank filled with water, whose temperature was kept constant by adding hot or ice water. In spite of this primitive technique, the variations of temperature never exceeded 0.5° in one and the same experiment. The flasks and tubes used in the apparatus were wrapped in black paper, the whole was arranged so as to move sideways by means of a shifting mechanism, and was covered at the top to secure complete darkness. Because some small amount of light could penetrate to the leaves by diffraction through the manometer tube, the external parts of the apparatus were covered with a sheet of black paper, which was only removed for the short periods necessary for reading. Before starting an experiment, the apparatus was allowed for 20 minutes or more to attain the temperature equilibrium.

Results of these experiments are given in the tables (I-III).

Table I

Group I (very young leaves). 22 whole leaves = 3.6 gm.

Temperature	c.mm. of O_2 consumed per hour per gram of fresh weight	Number of hours necessary to consume 1 c.c. O_2 per gram of fresh weight
16.4°	122	8.20
18.4°	128	7.81
22.0°	175	5.71
25.9°	233	4.29
29.9°	310	3.83
23.9°	195	5.13
10.8°	58	17.39
12.8°	99	10.08
7.6°	50	20.00
7.0°	49	20.41
3.0°	22	45.45

Table II

Group II (medium sized leaves). 3 whole leaves = 4.1 gm.

Temperature	c.mm. of O_2 consumed per hour per gram of fresh weight	Number of hours necessary to consume 1 c.c. O_2 per gram of fresh weight
14.3°	540	1.96
19.3°	820	1.22
24.4°	1300	0.72
15.9°	550	1.82
12.6°	270	3.70

Table III
Group III (oldest leaves).

(a) 3 whole leaves = 3.6 grm.; (b) 3 cut leaves = 3.55 grm.

Temperature	c.mm. of O ₂ consumed per hour per gram of fresh weight	Number of hours necessary to consume 1 c.c. O ₂ per gram of fresh weight
(a) 23.0°	393	2.55
31.0°	593	1.99
(b) 3.7°	146	6.85
8.0°	158	6.33
11.2°	200	5.00
15.3°	257	3.85
17.9°	282	3.53
20.0°	365	2.78
22.8°	374	2.68
26.0°	423	2.38

From these data temperature coefficients may be calculated, but any temperature coefficient, which has to serve as a basis of comparison, necessarily must really be a *constant*. It has been known for a long time that the temperature coefficient Q_{10} does not keep constant at different temperatures, nor does Arrhenius' "temperature increment," recently often applied to biological reactions (see Bělehrádek (4)). The reason why these thermochemical constants do not hold good for biological processes is probably the heterogeneous nature of the protoplasm and the high viscosity of its reacting phases. It may be shown that a large number of biological phenomena in animals and plants follow a rule, which has been established by one of us (Bělehrádek (2, 3)) as an empirical first approximation, but which is accurate enough for many cases. This rule is expressed by the equation:

$$y = \frac{a}{x^b} \quad \dots\dots(1),$$

where y is the time necessary to accomplish a given reaction (inverse of velocity), x the temperature in degrees centigrade, a and b constants, of which b is a true temperature coefficient, independent of temperature. In any case, where this formula holds good, the logarithms of time, plotted against the logarithms of temperature, give a straight line. The coefficient b may be calculated as follows:

$$b = \frac{\log y_1 - \log y_2}{\log x_1 + \log x_2} \quad \dots\dots(2).$$

From the graph (Fig. 1) it is evident that the experimental results are expressed with sufficient accuracy by the formula given

above (i). The lines for leaves of different age have different slopes and the temperature coefficients, calculated from these lines, vary accordingly. Thus in the group of leaves (i) $b = 1.11$, in (ii) $b = 2.18$, in (iii) $b = 0.88$.

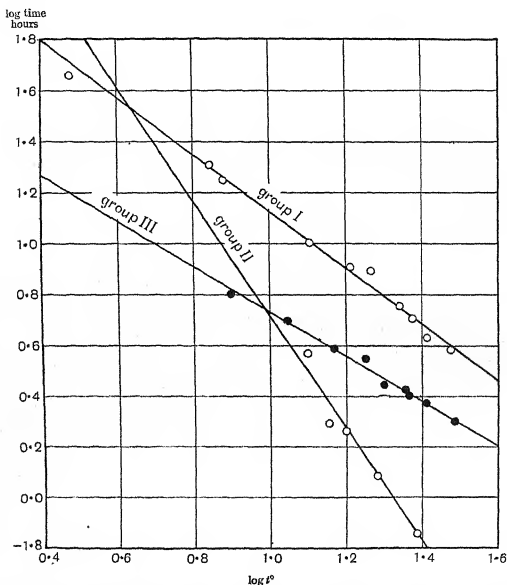


Fig. 1. Number of hours (in logarithmic values) necessary for consumption of a definite amount of oxygen, plotted against logarithms of temperature, gives straight lines whose slope is different for leaves of different age.

It is thus obvious that the action of temperature on the respiration of the plant depends on the age of the leaves and that plants form no exception to the rule which holds good for temperature coefficients in animals.

Variations of temperature coefficients with age might be ex-

plained in two alternative ways: either (1) the respiratory mechanism is governed by *chemical reactions* whose *nature* varies with the age; or (2) the rate of respiration is governed by the rate of diffusion, which again depends on the viscosity of the reacting protoplasmic phases; in this case it would be the *viscosity of protoplasm* which varies with age and which gives rise to variations of the temperature coefficient.

The first possibility seems to be excluded, because similar changes of temperature coefficients with age in animals are not sudden but continuous. For the rate of individual development of the beetle *Dytiscus semisulcatus* the temperature coefficient plotted against age gives a regular S-shaped curve (Bělehrádek (3, 5)). It is therefore more probable that the variations of temperature coefficients with age are due to a continuous change of protoplasmic colloids, which is accompanied by a continuous change of protoplasmic viscosity. It has been shown by more direct measurements that the protoplasmic viscosity generally increases with age (for bibliography see Weber (6), Bělehrádek (1)). In accordance with this fact the temperature coefficients of different physiological functions in animals mostly increase with age, but in some instances they vary in just the opposite direction.

In terms of this hypothesis, it seems that the viscosity of protoplasm—or, more exactly, the viscosity of reacting phases involved in the process of respiration—first increases with the age of the leaves, and that in the oldest leaves again a decrease occurs. This decrease might be explained by colloidal changes taking place in the cells (a) because of exposure to low temperatures during winter, or (b) because of the formation of spores. We cannot decide from our observations which of these two possibilities is the more probable.

The experiments described were performed in the Marine Biological Laboratory, Plymouth. We wish to express here our most heartfelt thanks to the Director of the Laboratory, Dr E. J. Allen, for his hospitality and for the interest he took in our work, and also our indebtedness to Professor Julian S. Huxley and to Dr C. F. A. Pantin for their valuable help and criticism.

SUMMARY

(1) Oxygen intake in leaves of *Scolopendrium scolopendrium* Karst. under varied temperature was measured and it was found that the temperature coefficient first increases and then decreases with the age of the leaves.

(2) Variations of temperature coefficient with age, found in these experiments, are analogous to those previously described in animals.

(3) These variations are explained by the hypothesis that colloidal changes occur in the protoplasm with age, and that these are accompanied by variations of the viscosity, and thus of the rate of diffusion, in the reacting protoplasmic phases.

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CHROMOSOME STUDIES.

I. RELATIONSHIP OF THE GENERA *ALSTROEMERIA* AND *BOMAREA*

By R. O. WHYTE

(With 38 figures in the text)

SOME cytological studies have already been made on the small group of the Amaryllidaceae, the Alstroemerieae, several species of *Alstroemeria* and *Bomarea* having been examined. Without going into their systematic position in full detail, it may be remarked that the group Alstroemerieae, found largely in Central America, northwards to Mexico, contains three genera, namely, *Alstroemeria*, with forty to fifty species, *Bomarea*, with over fifty species, and *Leontochir ovallei*, a monotypic genus reported not from Central America, but from Chile. The first two genera were at one time included under the one name, *Alstroemeria*, and according to Herbert (1837) they were separated by Mirbel for reasons which some botanists considered insufficient. These plants are used for greenhouse and outside decoration in England, about a dozen species of the two larger genera having been introduced at various times. Except for a specimen in the Herbarium at Kew, no reference to the existence of plants of *Leontochir ovallei* has been found, and applications to collectors and gardens have so far had no result. In the meantime, therefore, only material of the other two genera is available, but it is hoped that the search for the third may yet prove successful.

The early workers in cytology found the large nuclei and chromosomes of these plants suitable material for examination, and again in recent years, *Alstroemeria* has been used for the study of chromosome morphology, the somatic chromosomes being very useful in demonstrating the three main points upon which a distinction between chromosomes may be based, namely, size, position of fibre attachment, and the presence or absence of satellites. The previous contributions to the knowledge of chromosome numbers in this group may be summarised as follows:

	n	2n	
<i>Alstroemeria chilensis</i>	8	—	Strasburger (1882)
<i>Alstroemeria Pelegrina</i>	8	—	Guignard (1884)
<i>Alstroemeria pulchella</i>	8	—	Guignard (1889)
" "	8	—	Svensson-Stenar (1925)
<i>Alstroemeria brasiliensis</i>	—	16	Taylor (1926)
<i>Bomarea Caldasiana</i>	9	—	Svensson-Stenar (1925)

Guignard (1884) used *A. Pelegrina* for studies on the division of the cell nucleus, and in *A. pulchella* various stages of division of the egg cell are described in their relation to "les phénomènes morphologiques de la fécondation." The number of chromosomes is stated to be sixteen, but no critical analysis of shapes is given. Strasburger (1882) figures the meiosis in the pollen mother cell in side and polar views; except that an indication of a difference in size is to be seen in his Fig. 58, little idea is presented of the shapes of the respective chromosomes. Stenar (1925), in his studies on the embryology of the Alstroemerieae, found the chromosome numbers as noted above. Taylor (1926), used the genus *Alstroemeria* for his work on chromosome morphology; the results are considered in a later section.

METHODS AND MATERIAL

Fixations were made by the usual methods employed in the preparation of material for microtome sections, various fixatives being used, and also by the smear method. It was found that an incorrect picture of some stages of meiosis was presented in embedded material, and it is from the smear preparations that the greater part of the account of meiosis was taken. The recommended mixture for this method, a Flemming mixture, was used to some extent, but chrom-acetic or formalin-chrom-acetic (Karpechenko, 1927) was also found to give satisfactory results, both from a fixing and staining point of view. Gentian violet and iron-alum-haematoxylin were used for the microtome sections, gentian violet exclusively for the smears.

For fixations of root tips in the genera examined, Flemming solutions of various strengths and the formalin-chrom-acetic solution were used. Material fixed in Flemming and stained in haematoxylin gave the finer details (satellites, constrictions, etc.) in a satisfactory manner. Formalin-chrom-acetic, although fixing the cytoplasm well, tended to mask these details in the chromosomes when the same staining method was employed. Gentian violet, with either fixative, gave good preparations, but the requisite precision was in many cases lost.

Owing to the large size of the nuclei, microtome sections of anthers were cut at about 18μ , and root tips at about 12 – 14μ .

The plants from which the necessary material was collected were for the most part found in the Cambridge Botanic Garden; other material was obtained from the Royal Botanic Gardens at Kew and Edinburgh, and from the Botanic Garden, Glasgow.

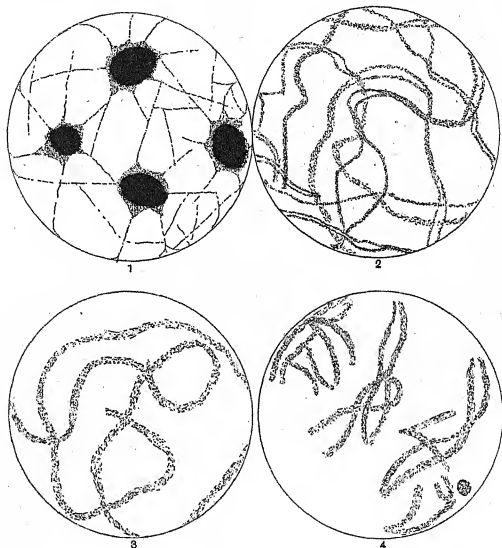
THE MEIOTIC PHASES

Meiosis in *Alstroemeria* and *Bomarea* does not differ in any way, and the following account refers therefore to pollen mother cell development in either genus.

The archesporial tissue before the beginning of prophase consists of tightly packed polygonal cells with large nuclei. The thin threads of the resting reticulum ramify and anastomose freely, while there may be seen in the general entangled network large chromatin masses, usually three or four in number (Fig. 1). These are presumably nucleoli acting as temporary chromatin reservoirs; they apparently take a prominent part in the metabolism of the nucleus during the interval that elapses between the last archesporial division and the beginning of the meiotic prophase, as the fine threads of the reticulum radiate out in all directions from the dense chromatic centres that these nucleoli form. In many examples it seems that there is no part of the periphery of these nucleoli that is not intimately associated with threads. The supposition that these chromatin reservoirs supply the threads with chromatin material is supported by the fact that, on completion of synizesis, only one such body remains, and this may still have a connection with the now regular spireme, as found by Latter (1926).

There is little contraction to be seen in the nuclei of the pollen mother cells during synizesis, this being found only in embedded material. During the early part of this stage the spireme undergoes a marked change, the ragged reticulum of the resting stage being replaced gradually by a continuous thread, much convoluted about the nuclear cavity. While this change is going on, the double nature of the spireme becomes noticeable (Fig. 2), but this is obscured again as the nuclei pass into the "open spireme" stage (Fig. 3). Newton (1926), studying the chromosomes of *Tulipa*, describes this first pairing of leptotene threads at synizesis, and notes the difference of opinion as to the value of the threads concerned; the "second split" described in *Tulipa* is assumed to be merely a repetition of the first, and subsequently there appears in each of the pairing members of

the bivalent thus formed the homotype split; this is said to be its first appearance. The spireme at "open spireme" stage in *Tulipa* may therefore be described as bivalent.



Figs. 1, 2, 3, 4. $\times 2200$

Fig. 1. Resting nucleus with prominent nucleoli.

Fig. 2. First pairing of the threads in meiotic prophase.

Fig. 3. Open spireme stage. No trace of doubleness in thread.

Fig. 4. Second pairing of the threads.

The figures are largely diagrammatic representations taken from camera lucida drawings.

In the *Alstroemeriacae*, it is difficult to see how the nuclei pass from the stage with the apparently continuous spireme to that with the pairing univalents (Fig. 4). The bivalent chromosomes that they form are similar in many ways to those figured by Newton (*loc. cit.*), but the homotypé split, forming the tetrad, does not become evident

in the bivalents of this group. The difficulty now arises as to whether we are to consider this pairing at diakinesis in the *Alstroemerieae* as the reappearance of the pairing of the leptotene thread at synizesis, while the homotype pairing has remained masked throughout, or whether in the earlier pairing we were seeing the somatic doubleness, which became obscured again during open spireme stage, and remained so during the formation of the bivalent chromosomes. Examination of the pollen mother cells of an F_1 generation hybrid in the genus *Bomarea* frequently shows that one of the nine bivalents is loosely paired, or in some cells quite unpaired (Fig. 11), this giving rise to the characteristic abnormalities to be noted in a later section of this paper. No marked disagreement is to be noted during the synizetic pairing, but at diakinesis a certain amount of incompatibility exists between the two homologous chromosomes concerned. One may therefore note the occurrence of eight normal bivalents and two rod-shaped univalents in the nucleus of one of these pollen mother cells. It seems conceivable that the first or somatic pairing has been completed successfully, and that this doubleness is present, though masked, in the unpaired univalents mentioned above. The heterotype pairing of these univalents is not, however, regularly achieved. In other words, it is held that non-conjunction is an expression of non-conjugation.

It is concluded, therefore, that in the *Alstroemerieae*, two distinct pairings are to be seen, but a different interpretation is suggested. The early pairing is found, in the plants examined, to represent a somatic pairing which becomes masked during the later stages when the formation of the bivalents takes place by a side-by-side arrangement of homologous threads. The thread in the open spireme stage is therefore univalent in character.

By progressive condensation and shortening, the chromosomes of the heterotypic division are formed. These show an assortment of shapes characteristic of the genus in which they occur; they will be considered in detail in the morphology section. At heterotype telophase all trace of the individual chromosomes is lost, and the nuclei of interkinesis have the appearance of a network of convoluted threads (Fig. 19). These latter have in some instances indications of a spiral structure. The formation of the cell walls in the pollen tetrads of the *Alstroemerieae* is successive, occurring at heterotype telophase and homotype telophase respectively.

CHROMOSOME MORPHOLOGY

Taylor (1926), studying the eight pairs of somatic chromosomes of *Alstroemeria brasiliensis*, noted that the genus *Alstroemeria* was among those plants with chromosomes of a large size, and that this genus supplied suitable material for the study of the fibre attachment zone. The present notes on these somatic chromosomes are largely a repetition of the results of Taylor's work, but as a comparison between *Alstroemeria*, with eight pairs of chromosomes, and *Bomarea*, with nine pairs, is intended, a full description of the various types will be given. The relation between somatic shapes and the form of

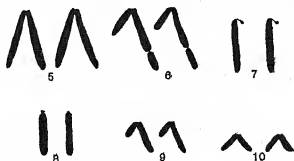
Figs. 5, 6, 7, 8, 9, 10. $\times 1100$

Fig. 5.	Somatic chromosome.	Type (a)
Fig. 6.	"	Type (b)
Fig. 7.	"	Type (c)
Fig. 8.	"	Type (d)
Fig. 9.	"	Type (e)
Fig. 10.	"	Type (f)

tetrad found in meiosis will also be dealt with. The plant used in this instance was *A. aurantiaca*, but the same remarks apply to *A. pulchella* and *A. haemantha*, and they agree with the account for *A. brasiliensis*.

The somatic chromosomes, studied in root tip mitoses, may be grouped into the following six classes:

Type (a). The largest chromosome pair, with fibre attachment near its centre. The ease with which the attachment zone can be seen is apt to vary with the fixative and stain employed (Fig. 5).

Type (b). This consists of a long shaft and a proximal lobe. The main shaft is a little longer than either half of type (a), and is not, as figured by Taylor, unsegmented throughout its length. A distal satellite can generally be demonstrated, both on the plate and in the metaphase of mitosis. It is apparently fairly constant in size (Fig. 6).

Type (c). A pair of rods with proximal satellites; these bodies are small, and are attached to the main shaft by means of a slender connecting thread (Fig. 7).

Type (d). Three pairs of rods, more or less equal in size. The short rounded attachment ends noted by Taylor can be seen in favourable preparations (Fig. 8).

Type (e). A pair of small chromosomes with a proximal lobe. The total length of the main shaft and the lobe is slightly less than that of the rods of type (d) (Fig. 9).

Type (f). A pair of small chromosomes with sub-median fibre attachment. The total length of both limbs is again slightly less than that of type (d) (Fig. 10).

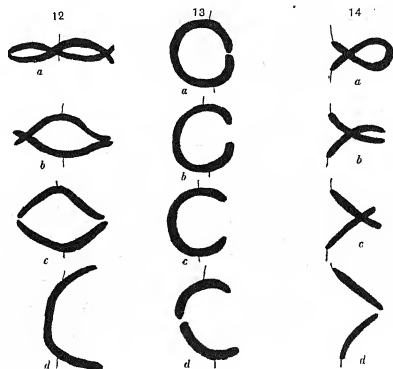


Fig. 11. Types of bivalent chromosomes at diakinesis (*Bomarea*). $\times 2200$.

The bivalent chromosomes of *Alstroemeria* are usually constant in the forms they assume at the heterotype division, and a study of the shape and size of these chromosomes, together with the method of separation of their components at heterotype metaphase, shows clearly which somatic pair is concerned in the formation of any given chromosome tetrad. The various forms of tetrads to be noted are found in smear preparations of a late diakinesis stage (Fig. 11), and the method of separation and the fibre attachments may be seen in microtome sections of an early metaphase, when a side view of part of the heterotype plate may be obtained.

The following types of bivalent chromosomes are to be noted in the meiotic divisions in the pollen mother cells of *Alstroemeria pulchella*:

Type A. A large double ring tetrad, sometimes forming a figure of eight, at other times having two free ends noticeable (Fig. 12 *a*). (The terms used to describe these tetrad forms follow as closely as possible the system adopted by Wilson (1925).) The fibre attachment is median or sub-median, and the method of separation is characteristic (Fig. 12 *b, c, d*). The relation of this chromosome to type (*a*) in the somatic series is evident, both being the largest of the complement and having a median fibre attachment.



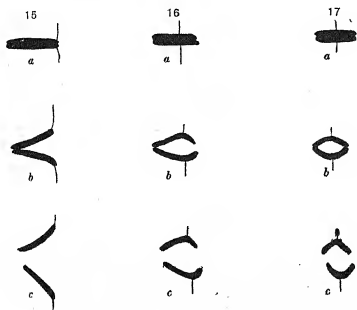
Figs. 12, 13, 14. $\times 2200$

Fig. 12.	Method of separation in bivalent chromosome.	Type A.
Fig. 13.	" " "	Type B.
Fig. 14.	" " "	Type C.

Type B. A ring tetrad, but without any evidence of free ends (Fig. 13 *a*). The fibre attachment is intermediate between median and terminal; thus on separation at metaphase, the "break" occurs first at a point near both attachment zones (Fig. 13 *b*), which point is usually situated on that side of the chromosome towards the centre of the plate. It is some little time before the second "break" occurs, causing the final separation of the univalent members in the form of asymmetrical V's (Fig. 13 *d*). This chromosome is conceivably the meiotic representative of type (*b*) (somatic), the relative size and the fibre attachment supporting this view.

Type C. The two univalent members of this tetrad are united only at one end and the two free limbs cross each other, forming a simple loop (Fig. 14 *a*). The attachment is sub-terminal; at the heterotype split, the two halves slide past each other, each being connected with the opposite pole instead of with that of its own side (Fig. 14 *b, c, d*). No trace of the satellite can be seen on this chromosome, evidently the representative of somatic type (*c*).

Type D. Three rod tetrads, more or less equal in size, very closely united as regards their univalent members and with terminal attachment (Fig. 15 *a*). Separation begins at the attachment end and



Figs. 15, 16, 17. $\times 2200$

Fig. 15.	Method of separation in bivalent chromosome.	Type D.
Fig. 16.	" " "	Type E.
Fig. 17.	" " "	Type F.

spreads along the length of the compact rod, opening it out into the shape of a wide V (Fig. 15 *b, c*). These rods are referable to the three pairs of rod-shaped chromosomes noted in the somatic nuclei.

Type E. A rod tetrad of approximately the same size as the previous rod types mentioned, but with sub-median fibre attachment (Fig. 16 *a*). This latter characteristic causes the appearance of asymmetrical V-shaped chromosomes at heterotype metaphase. The univalents composing this tetrad are in close connection, as was noted for type D, and the split commences almost at the centre of the rod, spreading towards both ends and naturally reaching completion consecutively (Fig. 16 *b, c*). The somatic chromosome represented in type (*e*).

Type F. Similar to the above, perhaps slightly smaller, and with true median attachment (Fig. 17 *a, b, c*), thus causing the appearance of small symmetrical V's at metaphase. Related to type (*f*) (somatic).

The somatic complement of eighteen chromosomes in the genus *Bomarea*, which has not been studied previously from the point of view of chromosome morphology, tends to be rather more difficult of interpretation than that of *Alstroemeria*, owing to the presence of an additional pair of chromosomes to further complicate an already involved equatorial plate. The nine pairs in *Bomarea* can be grouped in the same six classes as have already been described for *Alstroemeria* and the various chromosome types agree down to the minutest details. The distal satellites have been noted on type (*b*) somatic chromosomes of various species within the genus, and the small proximal satellite on type (*c*) is also present, similar in general size and method of attachment to the main shaft. The fibre attachments of the six types do not differ from those already noted in *Alstroemeria* and sufficient measurements have been made to show that the general length of the chromosomes or chromosome components is similar.

The increase in number is due to the presence of an extra pair of rod-shaped chromosomes of type (*d*), thus making four pairs in this group instead of three. The eight rods in a somatic nucleus of *Bomarea* are so much alike that it is impossible to make any distinction between them. It is therefore not possible to decide which is the additional pair.

As might be expected, the types of bivalents found in this genus are similar to those already described for *Alstroemeria*. The various tetrad forms are to be noted, and the attachments agree with the previous account. The increase in number from eight to nine is due to the presence of another rod tetrad, with terminal attachment, type D (meiotic).

INTERSPECIFIC HYBRIDS

Although the species of *Alstroemeria* are, according to reports received by the writer, capable of hybridising readily in their natural habitats, giving rise to an extremely varied group of individuals, presumably F_1 plants and their segregates, no reference has been found in the course of this investigation to the presence of authentic interspecific hybrids in this country. Some material collected at Cambridge, of doubtful parentage, showed suggestions of some of

the irregularities to be noted later in *Bomarea* hybrids, but beyond pointing out this suggestion of similarity, no stress will be laid on the result.

Hybrids between species of *Bomarea* have been made at different times at the Botanic Gardens at Cambridge and Glasgow. Although the parents were probably not reliable "pure line" material, they answer their respective descriptions tolerably well, and may therefore be considered as good species, as far as can be ascertained with such difficult material. The hybrids from which material was collected or supplied are grouped hereunder, the horticultural name of the F_1 generation plant being given on each occasion.

Bomarea Caldasiana by *B. edulis* = *B. cantabrigiensis*

B. Caldasiana by *B. patacocensis* = *B. Banksii*

B. Carderi by *B. edulis* = *B. Matthewsii*

B. edulis by *B. Carderi* = *B. Whittonii*

Most of the material was collected at Cambridge or Kew; for some material of the last two hybrids mentioned, the writer is indebted to Mr G. H. Banks, of the Glasgow Botanic Garden, who was responsible for making the crosses.

The first two hybrids, *B. cantabrigiensis* and *B. Banksii* (Lynch, 1914) are more or less intermediate as regards their vegetative characters, between their respective parents. The last two hybrids (Banks, 1926), are reciprocal crosses between two plants, one of which, *B. edulis*, is amongst the smallest species of the genus, while the other, *B. Carderi*, is one of the largest. The hybrids concerned are an example of matrocliny, each having a strong resemblance to its respective seed parent.

In the plants examined, the meiotic prophase is generally passed through without any abnormalities arising, and in many instances the actual heterotypic divisions are completed normally. In some divisions, however, one of the bivalents is loosely paired, or possibly not paired at all (Fig. 11), and the lagging of one or both of these univalents gives rise to characteristic irregularities at heterotype telophase, and in the ensuing homotype divisions. In addition to this occurrence of non-conjunction of homologous chromosomes, non-disjunction has also been noted. Examples of this are rare; both members of a bivalent chromosome go to the same pole, forming one nucleus with ten chromosomes and one with eight. As far as can be seen, it would appear that it is generally a rod tetrad that is associated with these irregularities.

In such cells, accessory nuclei formed by the alveolisation of a single chromosome are to be seen in the cytoplasm. In numerous instances, a triad instead of the normal pollen tetrad is present (Fig. 22), owing to the fact that one of the cells at telophase had nine chromosomes in the nucleus while the other had eight in the

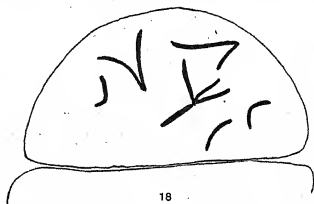


Fig. 18. Homotype plate. Note "homologous ends" of the four rods. $\times 2200$.

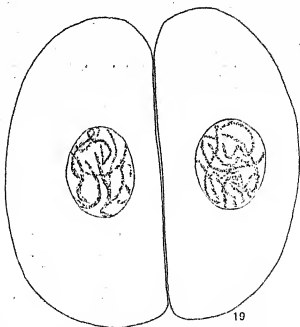


Fig. 19. Normal dyads. $\times 2200$.

nucleus and one in the cytoplasm. The latter cell does not always divide at the homotype division. On the other hand, both nuclei may divide normally (Fig. 23), as well as the small accessory nucleus formed by the lagging chromosome. In those pollen tetrads where both telophase nuclei have lost a chromosome, both the large nuclei may divide, and the lagging chromosomes may form separate spindles

in their respective cells; thus numerous different arrangements may be noted in the following telophase. In no tetrad has there been noted more than four tetrads.

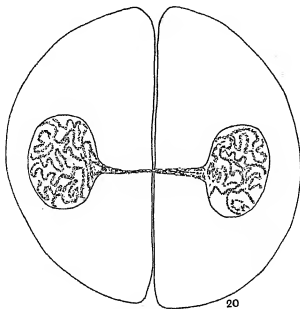


Fig. 20. Linking of the telophase nuclei following heterotype. $\times 2200$.

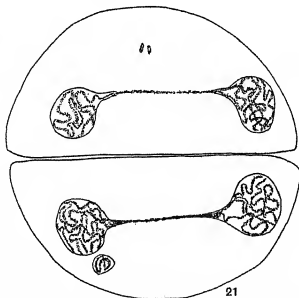


Fig. 21. Similar occurrence after homotype. $\times 2200$.

Another characteristic of hybrid cytology present in the *Bomarea* material is "linking" of the telophase nuclei after the reduction division (Fig. 20). This is present only in a mild form, the two nuclei at heterotype telophase being connected, sometimes across the

dividing wall of the dyad, by a strand of chromatin varying somewhat in thickness. This abnormality is regarded as related to the similar behaviour, in a more acute form, noted in semi-heterotypic

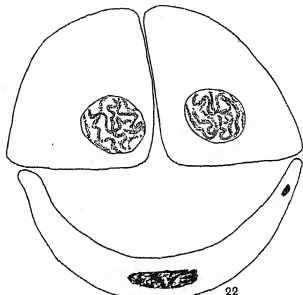


Fig. 22. Triad of two good cells and one abnormal. $\times 2200$.

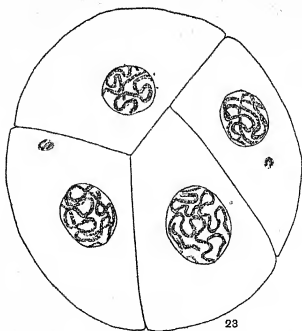


Fig. 23. Tetrad with accessory nuclei. $\times 2200$.

divisions in F_1 plants of certain hybrids, when the nucleus is reconstituted and the reduction division cancelled. Linking of the telophase nuclei following the homotype division is also to be noted in *Bomarea* (Fig. 21).

ALSTROEMERIEAE—GENERAL DISCUSSION

From the evidence presented in the foregoing sections of this paper, it will be seen that the two genera examined are closely related cytologically. The meiotic processes are similar in both and from the data supplied under the head of chromosome morphology, it may be said that this is another example of the cytological evidence confirming a close taxonomic relationship.

It does not appear necessary to deal at length with the numerous previous descriptions of chromosome pairing in the meiotic prophase. For this reason a comparison has been made only with the account of meiosis in the genus *Tulipa* (Newton, 1926); the fact that the comparison is made between two collections of material treated by the smear method adds to the value of the conclusions derived therefrom. It has already been noted that the accounts of chromosome pairing in the Alstroemerieae taken from embedded and smeared material respectively would be difficult to reconcile one with the other.

After the study of prophase in the F_1 generation hybrids, it is concluded that if the first pairing of leptotene threads at synizesis is the heterotype pairing, as stated by Newton, then some evidence of incompatibility should be seen in the "open spireme" stage. This, however, is shown not to be the case; on the contrary, incompatibility is noted after the second pairing. If we were to accept Newton's explanation for *Bomarea*, it would be necessary to assume that the homologous chromosomes paired completely at leptotene stage and that they later became antagonistic to each other. This explanation does not appear as suitable as that given in the meiosis section, where it is stated that the first pairing is a somatic pairing which becomes obscured completely until the homotype division, and that the second pairing forms the bivalents of the first, or reduction division.

Turning to the cytological relationship of the two genera, it is evident that in the genus *Bomarea* we have a $2n + 2$ form of the genus *Alstroemeria*. It may be argued that, on the other hand, *Alstroemeria* may be a $2n - 2$ form of *Bomarea*, but from the evidence available it would seem much less probable that a loss of chromosomes could have taken place. In the hybrid plants of *Bomarea* examined, any member of a pollen tetrad with eight chromosomes in the nucleus and one (accessory nucleus) in the cytoplasm had little chance of further development and in fact generally

disintegrated shortly after formation. The presence of non-conjunction, and on rare occasions of non-disjunction, of a single chromosome as a feature of the meiosis of hybrids has already been noted, and this offers attractive evidence towards a theory that *Bomarea* has arisen from *Alstroemeria* as a result of some such process. Natural hybridisation is common apparently in the latter genus in its wild state and the opportunity for the meeting of two $8 + 1$ *Alstroemeria* gametes, both arisen from an irregular division in which the result of the heterotype split may have been $7 + 2$ and 7 , may not have been actually very remote.

The aberrant chromosome arrangements in *Uvularia* (Belling, 1925) cause the appearance of gametes which might be supposed to form $2n + 2$ types; Clausen and Goodspeed (1924), however, find that the $2n + 2$ strains of *Datura* and *Nicotiana* are very inconstant. Their results are held to be "incompatible with the idea that permanent increase in chromosome number may result from non-disjunction." In this connection it is interesting to note that, just as the $2n + 2$ type of *Nicotiana* is super-enlarged, so is *Bomarea*, a possible $2n + 2$ type, actually a "super-enlarged" *Alstroemeria*.

The only conclusions that may safely be made from the evidence gathered in the cytological examination of the *Alstroemerieae* are as follows: (a) the genera *Alstroemeria* and *Bomarea* are identical as regards the meiotic processes in the pollen mother cells; (b) the somatic complement of *Alstroemeria* is $2a. 2b. 2c. 6d. 2e. 2f.$; (c) the somatic complement of *Bomarea* is $2a. 2b. 2c. 8d. 2e. 2f.$; (d) the study of the reduction processes in the pollen mother cells of the F_1 generation hybrids shows the presence of certain features, such as non-conjunction and non-disjunction of a single chromosome, which might be theoretically supposed to have played some part in the evolution of the *Bomarea* chromosome complement from that found in *Alstroemeria*.

SUMMARY

Meiosis. The resting nucleus preceding the meiotic prophase contains several large bodies, which may be considered as nucleoli or as derivatives of a single nucleolus by sub-division. At the conclusion of synizesis only one such body remains.

Lateral pairing of threads is noted at two stages of development. The first, during or about synizesis, is held to be the expression of the homotype of somatic split, which becomes obscured during the succeeding stages and does not reappear at the second pairing. This

latter is found to be the heterotype pairing, the univalent members of the bivalent chromosomes becoming arranged side by side.

The individuality of the chromosomes becomes obscured at interkinesis and during this stage the spiral structure of the threads is noticeable at times.

The formation of the dividing cell walls in the tetrad is successive.

Chromosome morphology. The present study confirms the description given by Taylor (1926) of the eight pairs of chromosomes of *Alstroemeria*, the only additional observation being that there is on type (b) a large distal satellite.

The types of somatic chromosomes are as follows: (a) Large V, equal arms. (b) Large V, unequal arms. (c) Pair of chromosomes with proximal satellites. (d) Three pairs of rods. (e) Small V, unequal arms. (f) Small V, equal arms.

The bivalent chromosomes or tetrads show clearly their relation to the somatic complement. The types observed are as follows: A. Large double ring tetrad. B. Large simple ring tetrad. C. Simple looped tetrad. D. Three rod tetrads, terminal attachment. E. Rod tetrad, sub-median attachment. F. Rod tetrad, median attachment.

The same types, somatic and meiotic, are found in the genus *Bomarea*. The increase in number ($2n = 18$) is due to the presence of another pair of rods (Type (d) somatic), which are similar to the others of the same type in every way.

Interspecific hybrids. The only irregularities found in the interspecific hybrids of *Bomarea* studied are non-conjunction and, rarely, non-disjunction of a rod tetrad. As a result, accessory nuclei are frequently formed by the lagging chromosome which has not been included in the nucleus. Linking of the telophase nuclei following heterotype and homotype divisions also occurs, but only in a mild form.

Literature will be found at the end of Chromosome Studies II.

CHROMOSOME STUDIES

II. INTERSPECIFIC HYBRIDS IN THE GENUS *NOLANA*

MATERIAL AND METHODS

The hybrids examined in the course of the present study were the four generations derived from the cross, *Nolana prostrata* L. by *N. atriplicifolia* Hort. A preliminary study of the cytology of the two parent species has already been made (Campin, 1925). No differences were to be found between the two species, the haploid chromosome number in each species being twelve. The present work confirms this result, the chromosomes at mitosis and meiosis being so small and so regular in size and shape that a suitable basis for comparison has yet to be found.

The material of the two parent species examined was drawn partly from pedigree individuals grown in pots and kept under glass, which were placed at the writer's disposal by Miss E. R. Saunders, and partly from plants cultivated in the open in a bed in the Cambridge Botanic Garden. The hybrid material was in all cases derived from crosses which had been made by Miss Saunders in the course of her investigations (not yet published) into the genetical inter-relations of these two species. Certain of the forms which made their appearance in the F_1 and later generations were selected for examination in the present investigation.

The 1927 fixations of anther material were made in a chrom-acetic solution, and as this gave consistently good results with iron-alum-haematoxylin, the same fixative was used in the 1928 collections in order that a correct comparison between the various plants might be obtained.

MEIOSIS IN THE POLLEN MOTHER CELLS

(a) *Parent species*

The following are the more important results of the previous study of the pollen mother cell development in the parent *Nolana* species (Campin, *loc. cit.*):

There is evidence of the formation of pro-chromosomes in the nucleus of the pollen mother cell. These appear as aggregations of chromatin on a delicate reticulum. Synizesis, which is a phase of considerable duration, is initiated by a contraction of this reticulum at one or more points and a progressive condensation of the

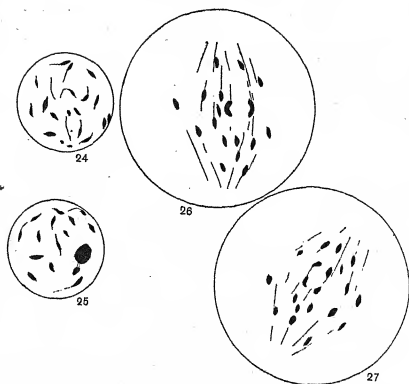
pro-chromosomes. The spireme emerging from synizesis is a single thread which throws out a number of loops into the periphery of the nuclear cavity. The sides of each loop come to lie alongside and twist about each other. Loops become detached and condense to form the bivalent chromosomes. It is concluded that the method of conjugation is by telosyndesis. Diakinesis is a phase of great precision, pairs of ellipsoidal chromosomes lying equally spaced on the periphery of the nuclear cavity. Divisions are quite regular and there is no loss of identity of the chromosomes in interkinesis; in the tetrad nuclei chromosomal aggregations are still clearly recognisable.

As already noted, the present study confirms the above observations, except in the case of the method of chromosome pairing; no definite evidence is available to support or contradict the view stated in the abstract. The bodies described as "pro-chromosomes" are very prominent in both the somatic and pollen mother cell nuclei.

(b) F_1 generation

Prophase in the pollen mother cells is passed through with perfect regularity until late in the open spireme stage. When the formation of the bivalents should normally begin, only a small amount of pairing is to be seen (Figs. 24 and 25), and at the time usual for diakinesis the nuclei of the pollen mother cells contain a number of rod-shaped chromosomes varying somewhat in length and thickness, with generally not more than three or four normal bivalents. Progressive condensation without any further pairing leads to the formation of many short univalents and the few bivalents. Dissolution of the nuclear membrane follows and the chromosomes are free in the cytoplasm. According to the amount of pairing that has taken place, different combinations may be found on the very irregular and scattered plates, e.g. four bivalents and sixteen univalents, three bivalents and eighteen univalents, etc.

As a result of the irregularity of the heterotype division (Figs. 26 and 27), the arrangement at heterotype and homotype telophase tends to be rather abnormal, the nuclei varying much in size according to the number of chromosomes they have received (Figs. 28 and 29). There are frequently more than four nuclei at homotype telophase, and as a result of these irregularities pollen fertility is very low, the counts of good and bad grains in a mature anther loculus giving something of the nature of 5-10 per cent. good pollen.

Figs. 24, 25, 26, 27. $\times 2200$.Figs. 24 and 25. Diakinesis in the F_1 generation.

Figs. 26 and 27. Irregular heterotype divisions. Few bivalents.

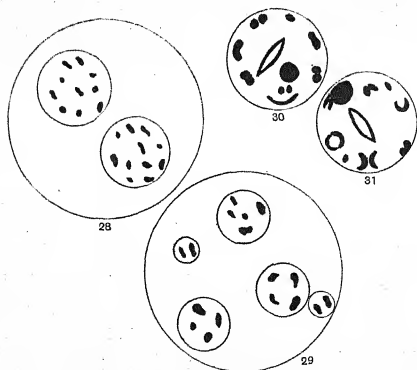
Figs. 28, 29, 30, 31. $\times 2200$.

Fig. 28. Fairly normal telophase nuclei.

Fig. 29. Telophase nuclei. Doubtful if after heterotype or homotype.

Figs. 30 and 31. Diakinesis in the F_2 generation.

(c) F_2 generation

In the plants of the F_2 generation derived by selfing from the almost completely sterile F_1 plants, a return to normal is very marked. The bivalents at diakinesis are regular and are of the usual *Nolana* shapes (Figs. 30 and 31), when these are distinctive. Plates give frequent counts of twelve, lagging univalents being few in number, seldom more than three or four (Figs. 32-35). The pollen fertility may be as high as 50 per cent. in favourable examples.

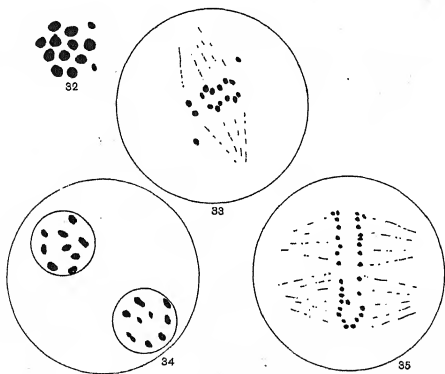
Figs. 32, 33, 34, 35. $\times 2200$.Fig. 32. Heterotype plate in F_2 plant.

Fig. 33. Reduction division in same plant.

Fig. 34. Regular interkinetic nuclei.

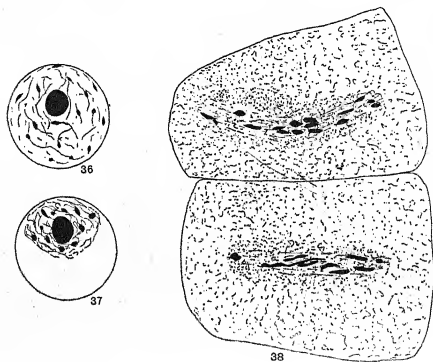
Fig. 35. Normal homotype division in F_2 plant.(d) F_3 and F_4 generations

The fertility of the F_2 plants is maintained, the divisions for the most part being regular.

A very marked abnormality was, however, noted in one plant selected as typical in the F_3 generation. The collection of material from this specimen was made in 1927, and before the following summer the plant had died; verification of the following results was therefore impossible. (It should be noted that only one flower was collected. As Miss Saunders has grown a normal F_4 generation from

the seed of this plant, it seems evident that the following account does not apply to the plant as a whole, but only to one abnormal shoot.)

Synizesis was reached with little appearance of anything unusual, except that the aggregations of chromatinic material along the spireme ("pro-chromosomes") were rather more prominent than in normal nuclei (Figs. 36 and 37). From synizesis the pollen mother



Figs. 36, 37, 38. $\times 2200$.

Figs. 36 and 37. About synizesis stage in the haploid flower.

Fig. 38. Abnormal reduction divisions in same flower. One spindle bent. Chromosomes of very irregular shapes.

cells passed direct to a division, omitting the usual open spireme stage and diakinesis completely. This division was extreme in its irregularity; the spindles were not present in their usual form, but were narrow, bent or otherwise deformed (Fig. 38). Chromatinic bodies, the counting of which was impossible, assorted themselves at random, no semblance of pairing was to be seen and the spindles disintegrated in many examples before this "division" was completed. No later stages are available. The value of these chromatinic bodies or chromosomes is doubtful, but they appear to be more of the nature of ordinary somatic chromosomes than of the univalents found in the pollen mother cells of the F_1 generation.

In consideration of these facts, and from subsequent examination of some somatic plates in the stylar tissue, there seems to be some basis for the conclusion that this is a haploid flower with a somatic chromosome number of twelve, and that the abnormal reduction division should be regarded in this light. The unfortunate loss of the plant makes any further conclusions impossible.

(e) *Nolana special type*

Observations were made on a form of *Nolana*, resembling *N. atriplicifolia* completely in its flower but differing from this species in habit. These plants do not show any cytological difference from the *Nolana* species already described, the haploid number being twelve and the pollen percentage being normal. As noted earlier, the somatic chromosomes do not give a good basis for comparison.

GENERAL DISCUSSION

With regard especially to the meiotic phenomena associated with the fact that hybridisation has taken place, the high degree of incompatibility is the most striking feature of the reduction processes in the F_1 generation. The direct correlation between degree of conjugation and the pollen fertility is also shown in the first two generations studied.

In the more favourable examples of pairing in the *Nolana* F_1 hybrid, four bivalents may be found and these may divide normally at heterotype division; the other unpaired univalents form with them an irregular collection of chromosomes at the centre of the cell, with no semblance of arrangement on an equatorial plate. The polar attraction must be assumed to be rather strong to cause this aggregation of chromosomes to pass to opposite poles in anything approaching equal numbers. The fact that the pollen fertility is of the nature of 5-10 per cent. is an indication that some regular telophase nuclei must be formed as a result of reduction in the F_1 generation. The return to a normal diakinesis in the F_2 generation, with the subsequent formation of ten or more bivalent chromosomes on the plate, is associated with a corresponding marked increase in the pollen fertility, this figure having risen to 50 per cent. It is conceivable that the two parental sets of twelve chromosomes have either lost their first antagonism towards each other, or have become adapted to what was an unfavourable cytoplasmic environment, whichever may be assumed to be the cause of the incompatibility in the first generation.

The occurrence of the haploid flower reported in an F_3 plant has probably little or no connection with the previous upheavals caused by hybridisation, for the production of good seeds capable of giving rise to an F_4 generation may be taken as an indication that the plant concerned was actually diploid. The appearance in the last growing season of a haploid flower, probably growing on a haploid shoot, may be connected with the enfeebled condition of the plant at the time. It is therefore not directly comparable with the haploids of *Solanum* (Jorgensen, 1928), *Nicotiana* (Clausen and Mann, 1924), *Datura* (Belling and Blakeslee, 1923) and *Triticum* (Gaines and Aase, 1926), although the type of the actual reduction division is on the whole similar, with a tendency towards a more extreme type of irregularity. The occurrence of a haploid shoot and flower on a diploid plant is probably more analogous with the origin of *Primula Kewensis* $4n$ from a tetraploid part of a normal diploid plant (Newton and Pellew, 1929).

The term "percentage pollen fertility" has been used at various stages in these notes. There seems to be no general agreement as to when the estimation of "good" and "bad" pollen should be made. A germination estimation gives one some idea of the general degree of sterility, but it probably would not agree in all examples with an estimation taken from an undehiscent anther, between the "good" grains on the one hand and the "bad" grains and immature pollen grains on the other. Further, in *Nolana* species and hybrids, an estimation at an earlier stage than the above, when the anther loculi contain only immature pollen grains, would in some instances indicate a 100 per cent. sterility count. This discrepancy is due to the operation of a physiological factor; the unnatural habitat in which the plants are growing tends to be expressed in tapetal failure at various stages of pollen mother cell development. This aspect of the case, which is comparable to some extent to the "time factor" (Whyte, 1929 *a, b, c*), will be dealt with in detail in a later paper. In the meantime, it is suggested that three estimations of fertility might be given; (*a*) at the time when the separate members of the pollen tetrads are about to change to mature pollen, (*b*) before a mature anther has dehisced, and (*c*) a germination estimation. In the present paper, the second figure is the one given, but this is taken only from examples where the influence of the physiological factor is found to be absent.

The writer wishes to express his sincere thanks to Miss E. R. Saunders for placing her *Nolana* material at his disposal, and to

Mr F. T. Brooks for his valuable help and advice during this and other studies.

SUMMARY

The hybrids examined were the four generations derived from the cross, *Nolana prostrata* L. by *N. atriplicifolia* Hort., which had been made by Miss E. R. Saunders in the course of her investigations (not yet published) into the relations of the two species.

Non-conjunction in the F_1 plants is marked, only three or four of the possible twelve bivalents being formed. The remaining univalents are distributed to opposite poles in a fairly regular manner. As a result of these divisions, about 5-10 per cent. good pollen is formed.

Diakinesis in the F_2 plants has returned to the normal, ten or more bivalents occurring regularly. Divisions are more regular and the pollen fertility is more of the nature of 50 per cent.

A haploid flower, with a very abnormal meiotic division, was found in an F_3 plant. As, however, good seed had been collected previously and a normal progeny raised, it is assumed that the plant was originally diploid and that the haploid flower was an abnormality arising in the last growing season. Otherwise the divisions in the F_3 plants were normal.

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THE YOUNG LEAF AS THE INHIBITING ORGAN

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(With 1 figure in the text)

I. INTRODUCTION

IN describing experiments on the correlative inhibition of axillary buds in seedlings, it has so far been usual to refer to the inhibiting region by the rather vague expressions "tip" or "apex of the shoot" (1, 9, 12, 13), which naturally include the young leaves of the terminal bud as well as the stem apex. But it is clearly desirable to determine exactly what part of the apex of the shoot it is that inhibits.

In older plants, of certain species, it has been found that other parts, such as the larger leaves, also inhibit. Thus Goebel (6), pp. 809, 810) succeeded in causing the axillary buds of various trees to grow out by defoliating the shoots without removing the apex. He found however that the terminal bud also inhibits to some degree. This effect of defoliation has since been several times confirmed, for instance by Sandt (11), p. 122) and by Dostál (5), p. 441). The latter considers that in trees the leaves inhibit not only the axillary buds, but also the terminal buds, causing them after a time to stop elongating and to turn into "winter buds."

Dostál (3) found also that in species (mostly herbaceous) of fifteen different orders, the expanded leaves partially inhibited their own axillary buds. For after removal of the apex, the leaves prevented their axillary buds from growing out as rapidly as buds whose subtending leaves were removed. The leaves inhibited only if they were allowed to photosynthesise or were nourished in some other way.

In a later valuable paper (4), Dostál has reported a very thorough investigation of this inhibiting effect. As to its nature he has reached the same conclusion as before (3), p. 533 and (4), p. 133), namely, that it is brought about by some substance or substances which are produced in the metabolism of the leaves. It may be noted that if

one leaf of an opposite pair is removed, its axillary grows out more rapidly and soon begins to inhibit strongly the axillary of the remaining leaf (3, pp. 88-9). Consequently the direct inhibiting effect of a leaf on its own axillary need only be a slight one, just sufficient to turn the balance of vigour in favour of the opposite axillary.

Loeb (8) found that in *Bryophyllum* also the foliage leaves inhibit, as well as the growing buds.

It had for some time seemed desirable to determine exactly the extent of the apical inhibiting region in the specially convenient seedlings of Leguminosae. But the question was placed in a new light through an observation made in another connection in the Oxford botanical laboratory by Miss C. M. Pilkington, who noticed that in seedlings of *Vicia Faba* the buds begin to grow out if the leaves are removed from the shoot, from below upwards, to those of small size, although the apex of the stem is not injured. From this observation, which suggested that in these seedlings the inhibition coming from the apex of the shoot may really come from its leaves, the present investigation starts. The question of the nature of inhibition has been considered previously, and is here not further discussed.

2. EXPERIMENTAL ARRANGEMENTS

Except where otherwise stated, pea seedlings were used of a race named "Thomas Laxton." Peas had the advantage that their axillaries grew out, in controls in the same conditions, at rates which were surprisingly uniform, provided that only plants with perfectly healthy cotyledons were used. Care was taken to see that the cotyledons were still not nearly exhausted at the end of the experiments.

The axillaries of the first leaves were selected for measurement. In the plants used, they were at the start rather uniformly about 1 mm. long. The higher buds were removed as soon as they could be seen. The axillaries never grew out in intact seedlings, except in a very few that were visibly unhealthy. The plants were grown in a greenhouse, and the operations were performed on batches of similar plants on the same day, or occasionally, in steady weather, with an interval of 1 or 2 days between them. It is therefore possible to compare experiments and controls of each batch, but not those of different batches, as the temperature often varied greatly.

The leaves of the pea are in two ranks (distichous), and between the lengths of 1 and 20 mm., each leaf is about three times as long

as the next youngest. In order to simplify the explanations, the term "growth-interval" will be introduced. By this will be meant the period of time taken by a leaf to grow to the size that was possessed by the next largest leaf at the beginning of the period. This time is roughly the same for all leaves: it varied from 2 to 5 days according to temperature.

If the leaves are removed up to a young leaf of length " x " inclusive, and then successive leaves are removed in turn at the end of their growth intervals, the largest remaining leaf will be always somewhere between a length of about $x/3$ and x . This was the procedure adopted in the experiments with terminal buds. The apices were protected with cotton-wool when necessary.

Controls were provided by decapitating seedlings of the same batch in the first internode, and removing their first leaves.

The figures given for the size of leaf in each experiment are means. The sizes in individual plants varied to not more than 35 per cent. from the mean in the experiments with terminal buds, and not more than 17 per cent. in those with single leaves. The leaves were measured to the end of a pinna.

3. PRELIMINARY EXPERIMENTS

It was soon found that in young seedlings of *Pisum sativum* the axillaries of the first leaves could indeed be made to grow out by removing the leaves from the stem, from below upwards, to those of about 2.5 mm. inclusive. In seedlings of *Vicia Faba*, the first leaf axillary of the main shoot, which is a very large bud and grows out very readily, grew out slowly when the leaves were removed only so far as to those of 8 or 10 mm. Also in two seedlings of *Phaseolus multiflorus* the axillaries of the cotyledons were made to grow out rapidly by removing the leaves up to those of 1 or 2 mm. inclusive, and also removing the higher buds. Yet in all these plants, the apices of the stems and the very small remaining leaves were not injured, but went on growing.

In order to keep the axillaries growing indefinitely, it was necessary to keep on removing the largest leaves of the terminal bud after their growth-intervals. When this was no longer done, the growth of the axillaries was before long completely stopped again, as was observed in several pea seedlings. It must be concluded that at least a considerable part of the inhibiting effect of the intact shoot comes from some of its leaves that have reached more than a certain small size. When these are removed, the axillaries grow out until

the remaining very small leaves near the apex grow to the necessary size and inhibit them completely again.

It may indeed be suggested that the inhibition comes not from the leaves themselves, but from those processes of growth in the stem below which depend on them. For as shown by Jost(7), the formation of leaf traces and the growth of the cambium in stems often depend on the presence of growing leaves above. But even if this suggestion were correct, it would only be equivalent to saying that the leaves inhibit not directly but indirectly, through the changes which they provoke in the stem. Thus it would still be permissible to say, for simplicity, that the leaves inhibit. The elongation of the stem will be considered below.

It must be noted that McCallum ((8), p. 248) defoliated growing shoots of several woody plants, chiefly *Salix*. He cut away "not only the larger leaves, but even those still folded in the bud." Yet the axillaries did not grow out, unless the "tip of the shoot" was also removed. It is possible, however, that the axillaries might have grown out, if he had removed the leaves to a smaller size still, or if he had kept on removing them after their growth-intervals.

The writer defoliated a young elongating shoot of the current year, of *Salix fragilis*, in May, leaving the terminal bud with largest remaining leaf of 5 mm. The shoot was left in place on the bush. After about a month, two axillary buds of this shoot had swollen up, and begun to expand their leaves, although the terminal bud had meanwhile continued to grow actively. No other axillary bud on the whole bush showed any sign of starting to grow.

It therefore does not seem possible to rely on these negative results of McCallum, which seem moreover to be in conflict with the results of Goebel ((9), p. 809).

4. SHOWING THAT THE STEM APEX DOES NOT INHIBIT

The next point to be determined was whether the stem apex and youngest leaf rudiments, or the other parts of the stem also inhibit to some slight degree. For this purpose, it was necessary to find whether in seedlings from which the leaves were removed up to those of some very small size, the axillaries would grow out fully as rapidly as in the decapitated controls.

Exp. 1. In six pea seedlings, the leaves were removed from the stem until the largest remaining leaves (which were at first the seventh leaves from the base) were 0.75 mm. long. They grew to

length 1.5 mm. in a growth-interval, and were then removed in turn. The height of the seedlings was at first about 35 mm.

In 3 days, the axillaries of the first leaves grew:

In 6 experiments, 6, 4.5, 3.5, 5, 4 and 6 mm. Mean = 4.8 mm.

In 6 controls, 4, 4, 3.5, 5, 3.5 and 5.5 mm. Mean = 4.2 mm.

The apices of the shoots, in this and subsequent experiments, were examined some days later and found to have gone on growing actively.

This experiment was performed on a very uniform batch of seedlings, but it shows no significant difference in the rates of outgrowth of axillaries in experiments and controls. The very rapid growth was due to hot weather, with temperatures up to 27° C.

The same result was obtained in the two following earlier experiments, which were however made on less uniform batches of seedlings, and so show more variation in the rates of growth.

Exp. 2. Arrangement as in *Exp. 1.* The largest remaining leaves grew from length 1 to 2 mm. in a growth-interval. Height not less than 25 mm.

In 7 days, the axillaries grew:

In 3 experiments, 6, 4.5 and 3.5 mm. Mean = 4.7 mm.

In 3 controls, 7, 4 and 3.5 mm. Mean = 4.8 mm.

Exp. 3. The largest remaining leaves grew from length 0.5 to 1 mm. in a growth-interval. Height not less than 25 mm.

In 7 days, the axillaries grew:

In 4 experiments, 4, 8, 4 and 4 mm. Mean = 5 mm.

In 4 controls, 4.5, 3, 4 and 4.5 mm. Mean = 4 mm.

From *Exps. 1* and *2* it can be seen that the apex of the stem, together with the youngest leaves down to a leaf of length varying from 0.75 to 1.5 mm. or from 1 to 2 mm. in a growth-interval, does not have any inhibiting effect strong enough to be detected in the conditions of the experiments. If it inhibits at all, it can only do so extremely slightly. As to the young leaves, it may safely be concluded that at a length of 1 mm. they have not yet begun to inhibit appreciably. There are then present four other still smaller leaf rudiments.

In *Exp. 3* also there was no inhibition, but the largest remaining leaf was smaller.

The internodes below the terminal buds continued to elongate fairly rapidly in the defoliated shoots of *Exp. 1*, though distinctly less rapidly than in intact shoots. Since absolutely no inhibition

was detected, it follows that the elongation of the stem does not inhibit either, or not more than very slightly if at all.

5. INHIBITION BY THE TERMINAL BUD

The next question was at what size the young leaves begin to inhibit. The following experiment throws light on this question.

Exp. 4. Arrangement as before. The largest remaining leaves (at first the seventh) grew from length 1.25 to 3.25 mm. in each growth-interval. Height 60 mm.

In 6 days, the axillaries grew:

In 5 experiments, 2.5, 2.5, 2.25, 4.5 and 4 mm. Mean = 3.2 mm.

In 6 controls, 10, 11, 8, 11, 12.5 and 11 mm. Mean = 10.6 mm.

The ratio of the means is 30 : 100.

From this result, it is clear that when the largest remaining leaf is growing from length 1.25 to 3.25 mm. in each growth-interval, the terminal bud inhibits strongly enough to slow down the growth of the axillary very greatly, though not to stop it entirely.

If the leaf were inhibiting only at the very end of each growth-interval, it would not have time to delay the axillary very much. Consequently it may safely be concluded that the leaf has already begun to inhibit at a length of 2 or 2.5 mm.

Next it must be asked, to what size the leaves must remain, in order that the terminal bud may inhibit completely. The following experiment is relevant.

Exp. 5. Arrangement as before. The largest remaining leaves grew from length 4.5 to 11 mm. in each growth-interval. Height not less than 25 mm.

The axillaries grew:

In 2 experiments, in 6 days, 0.75 and 0.5 mm.,

and in 10 days, 0.75 and 0.5 mm.

In 4 controls, in 6 days, 4.5, 3, 4 and 4.5. (Mean = 4 mm.)

and in 10 days, 15, 10, 11 and 16.5. Mean = 13 mm.

Thus the terminal bud inhibits the axillary almost completely, when its largest leaf is growing from length 4.5 to 11 mm. in each growth-interval. It may safely be concluded that a terminal bud with largest leaf of about 7 mm. long is inhibiting almost completely. At this stage, it has two leaves large enough to be inhibiting.

6. THE EFFECT OF AGE AND HEIGHT

At this point, it is necessary to refer to a complicating factor, which proved very puzzling until it was detected. The strength of inhibition is not constant for any given size of young leaf, but *increases* with the age of the seedlings and consequently with the height to the leaf. In very young seedlings, only 20 mm. high, inhibition was much weaker, as is shown by the two following experiments.

Exp. 6. Arrangement as before. Height 20 mm. The largest remaining leaves (at first the fifth) grew from length 3 to 9 mm. in a growth-interval.

In 7 days, the mean growth of axillaries was:

In 6 experiments, 4.0 mm.

In 5 controls, 10.4 mm.

The ratio of the means is 38 : 100.

Exp. 7. Height 20 mm. The largest remaining leaves (at first the fifth) grew from length 3 to 7.5 mm. in each growth-interval.

The mean growth of axillaries was:

In 7 experiments, in 7 days 2.8 mm.; in 11 days 8.7 mm.

In 9 controls, in 7 days 3.7 mm.; in 11 days 16.6 mm.

The ratio of the means after 7 days is 76 : 100.

Thus in the very short seedlings of Exps. 6 and 7, the terminal buds inhibited rather less strongly than in those of Exp. 4, which were three times as tall, but had largest leaves only half as long.

From the height, the distance from terminal bud to axillary can be roughly calculated by subtracting from 6 to 12 mm. for the epicotyl, according to age.

This surprising effect of age and height, which it is hoped soon to investigate further, was found even more strongly in later experiments with single leaves reported below. Naturally with age there vary many other factors besides height, and consequently it cannot yet be concluded that inhibition really increases in strength as it travels down a stem. There may very probably be some quite different explanation, such as that in older seedlings the axillaries have perhaps less inherent tendency to grow out. However, Child (1, 2) has considered provisionally that in *Phaseolus* inhibition, after being weakened in passing a partial physiological block, increases in strength again as it travels down the stem. But his results have been

published only in summarised form. Mogk also (10), p. 593) has found that certain shoots of *Vicia Faba* inhibit more strongly as they grow longer.

7. INHIBITION BY SINGLE LEAVES

It may next be asked how the inhibiting effect of a leaf varies with the successive stages of its growth up to full size. This could not be determined by continuing the experiments with terminal buds: for when the largest leaf of the bud is 6 mm. long or more, then the next smaller leaf has also begun to inhibit, so that their effects cannot be studied separately. It was therefore necessary to work with single leaves, removing all the leaves below them and decapitating the shoot above them. The single leaves had to remain growing all the time, but the duration of the experiments was made rather short. One of the stipules was removed in order to make the decapitation easier.

For an exact comparison the leaves of different sizes should be compared at equal heights (or possibly in seedlings of equal age and height) on account of the height-effect. For this reason, later-formed leaves were used for the small sizes. But even so, the heights varied considerably, so that from the following six experiments (which are supported by three or four others unpublished) only a very rough comparison can be made. The full length finally reached by the fifth leaves was from 40 to 45 mm. and the sixth and seventh leaves were nearly similar.

Exp. 8. In 4 days, the leaf (the seventh) grew from a length of 2.75 to one of 7.5 mm. Height to leaf at the start 80 mm.

The axillaries grew:

In experiments, 2, 0.5, 3 and 0.75 mm. Mean = 1.6 mm.

In controls, 3, 3.5, 3.5 and 3 mm. Mean = 3.3 mm.

The ratio of the means is 48.5 : 100.

Exp. 9. In 3 days, the leaf (the sixth) grew from 3.5 to 10.5 mm. Height to leaf 55 mm.

The axillaries grew:

In experiments, 1, 0.75 and 1 mm. Mean = 0.9 mm.

In controls, 3, 4, 3, 2, 3, 3.5 and 3 mm. Mean = 3.1 mm.

The ratio of the means is 29 : 100.

Exp. 10. In 4 days, the leaf (the fifth) grew from 9.5 to 25.5 mm. Height to leaf 42 mm.

The axillaries grew:

In experiments, 0.5, 1, 1.5, 2.5 and 1 mm. Mean = 1.3 mm.

In controls, 3, 2.5, 3.5, 3, 4.5, 3 and 3 mm. Mean = 3.2 mm.

The ratio of the means is 40.6 : 100.

Exp. 11. In 5 days, the leaf (the fifth) grew from 14 to 36.5 mm.
Height to leaf 50 mm.

The axillaries grew:

In experiments, 0.5, 0, 0.25, 0.5, 1.5 and 0.25 mm.

Mean = 0.5 mm.

In controls, 3.5, 3.5, 3.5, 4 and 4 mm. Mean = 3.7 mm.

The ratio of the means is 13.5 : 100.

Exp. 12. In 5 days, the leaf (the fifth) grew from 20 to 36.5 mm.
Height to leaf 70 mm.

The axillaries grew:

In experiments, 3, 1.5, 3, 3, 2.5 and 2.5 mm. Mean = 2.6 mm.

In controls, 4.5, 3.5, 3.5, 3, 3.5, 5.5 and 3.5 mm.

Mean = 3.9 mm.

The ratio of the means is 66.7 : 100.

Exp. 13. In 3 days, the leaf (the third) grew only from 24.5 to 27 mm., having then reached its full size, which is smaller than that of the higher leaves. Both stipules remained. Height to leaf, 42 mm.

The axillaries grew:

In experiments, 2, 2.5, 3, 3.5, 3, 3 and 3.5 mm.

Mean = 2.9 mm.

In controls, 3, 4, 3, 2, 3, 3.5 and 3 mm. Mean = 3.1 mm.

The ratio of the means is 93.5 : 100.

To these results may be added those obtained, at height 60 mm. in *Exp. 4*, in which the terminal bud was present, but only its largest leaf, of mean length 2 mm. during its growth-interval, was inhibiting. The ratio of the means in *Exp. 4* was 30 : 100.

It is possible, however, that the leaves of 1 mm. and less, though they do not by themselves have an inhibiting effect strong enough to be detected (*Exps. 1* and *2*), may yet have a very slight effect which when added to that of the 2 mm. leaf serves to increase it. This experiment therefore cannot be strictly compared with those with single leaves, but still it provides an upper limit and an approximation for the effect of the 2 mm. leaf.

From the results of *Exps. 8* to *13*, it can be seen that, in the conditions of these experiments a single leaf never or scarcely ever

inhibits completely, so that in the normal shoot the complete inhibition must be due to several leaves acting together. But the partial inhibiting effect of a single leaf at the different sizes can be conveniently measured by the ratios of the growth of axillaries in experiments and controls given above.

The results may be summed up as follows. The leaf has begun to inhibit partially at a length of 2 or 2.5 mm. (Exp. 4), and it continues to do so, without any very great change in intensity, when growing from length 2.75 to 7.5 mm. (Exp. 8), and at successive stages up to that at which it grows from length 14 to 36.5 mm. But when it is growing from 20 to 36 mm. the inhibition has greatly diminished (Exp. 12), and when the leaf is within 2 or 3 mm. of its final size, it has practically disappeared (Exp. 13).

If allowance is made for the differences in height, it appears probable that the inhibiting effect increases slowly from length 2 mm. up to the maximum of Exp. 11, in the stage from 14 to 36.5 mm. But this maximum, if it is genuine, probably comes at a length between 14 and 20 mm.; for from Exp. 12 it appears that inhibition must begin to fall off again rather rapidly soon after length 20 mm.—that is, about when the leaf reaches half its full length.

By similar experiments it was found that in *Vicia Faba* also a single growing leaf inhibits partially, and that in *Phaseolus multiflorus* the first pair of leaves does so. In both species the leaves continue to inhibit throughout most or perhaps all of their period of growth.

In other experiments with *Pisum*, in which the small fifth leaves of very short seedlings were used, the "height-effect" was very striking. Three such experiments were performed (Exps. 14, 15, 16), with four or five experimental plants and five or six controls in each. The heights were only 20 or 15 mm. The single remaining leaves grew from length 3 to 8.5, from 3.25 to 11, and from 2 to 7.5 mm. in the three experiments respectively. The ratios of the mean growth of axillaries in experiments and in controls were 96 : 100, 95 : 100, and 105 : 100.

Thus at this height, the leaves did not inhibit appreciably. The results should be contrasted with those of Exps. 8 and 9, which show that leaves of about the same size inhibit strongly at heights of 60 and 80 mm. The height-effect was obvious in several other experiments also.

8. DISCUSSION

It had previously been pointed out by Dostál (3), p. 553) that inhibition by leaves seems to be essentially similar to the well-known inhibition by the terminal bud. The present investigation shows that inhibition by the terminal bud simply is an inhibition by leaves, namely by the young leaves of the bud.

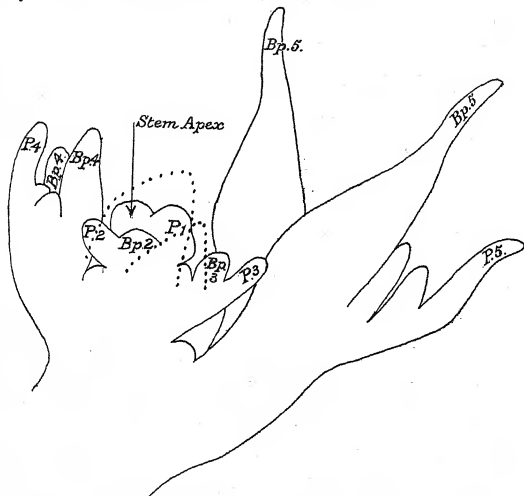


Fig. 1. Stem apex and five leaf primordia of a pea seedling. Drawn under camera lucida $\times 60$. Explanation in text.

Lettering. *P* = rachis of primordium. *Bp* = basal pinna.

The stem apex and leaf primordia of 1 mm. or less were found, by themselves, not to inhibit appreciably. But nonetheless it is possible that they may really have a slight inhibiting effect which, in proportion to their mass, is as strong as that of the larger leaves or even stronger. If so, then the reason why this effect does not show itself would be simply that their mass is too small.

The accompanying figure makes it easy to see how rapidly the sizes of the successive leaves diminish. It shows the apex of a young

pea seedling with the five youngest leaf primordia, cleared in potash, mounted entire, and lying in the plane of the leaves. The primordia are numbered downwards from *P* 1 to *P* 5. The stipules of *P* 4 and *P* 5 have been removed, and the uppermost stipules of *P* 2 and *P* 3 are shown by dotted lines, those on the lower side being omitted.

The largest of the five primordia shown is 1.4 mm. long, and even this one has scarcely begun to inhibit (Exp. 1). The actual stem apex above the primordia is so small that it could hardly be expected to inhibit. It measures only 135 μ across and 100 μ downwards. Yet the stem apex of the pea is an exceptionally large one. In another seedling, it was found to measure 170 μ across and 135 μ down, being then more nearly at the end of a growth-interval, or "plastochron."

The leaves continue to inhibit strongly until they reach about 20 mm., or nearly half their final length. At this stage, their cells have long ceased to appear embryonic, although they were found to continue dividing actively even later than this. For on comparing a leaf of 22.5 mm. with one of the full length of 45 mm., it was found that the length of a pinna increased by 80 per cent. while the diameters of the cells of its "palisade" layer increased in the same direction by only 25 per cent. Thus even during the second half of the elongation of the leaf, many cells are dividing. Measurements on another pair of leaves confirmed this point.

In Dostál's plants, the inhibiting leaves must often have been full-grown, or nearly so, and Mogk states ((10), p. 651) that in *Salix* full-grown shoots inhibit, but that in the seedlings of Leguminosae only growing shoots do so. But it might be suspected that inhibition by full-grown parts was different in nature from inhibition by terminal buds, and it was partly for this reason that it seemed of interest to follow the inhibiting effect of the leaf of *Pisum* from the earliest stages onwards. Since its inhibiting effect did not change very greatly in strength between the lengths of 3 and 20 mm., it is natural to suppose (unless evidence to the contrary is produced) that, in the seedlings of Leguminosae at least, the inhibiting effect of the leaf is in the main of the same nature at all stages.

The elongation of the stem was found not to inhibit appreciably, but the formation of leaf traces may do so, as was pointed out in section 3. However the leaf trace is, as Jost puts it ((7), p. 545) "physiologically part of the leaf," and here it has been so considered.

Finally the results show that to speak of the "apex of the shoot" or "terminal bud" as the inhibiting region is, for the pea at least, not so very inaccurate after all. For almost the whole inhibiting effect of the shoot comes from three of its leaves—namely from those that are between the lengths of 2 and 6 mm., of 6 and 18 mm., and of 18 and 30 or 40 mm. respectively in each growth-interval. And of these three leaves, two still form part of the terminal bud, while the third is only a very little way below it.

After this paper had been completed, Professor Dostál kindly sent from Brunn several interesting papers by himself and others on correlation. One point in one of these, by Weiskopf (14), must here be briefly considered. Unfortunately only the summary is in French, the remainder being in Czech. Weiskopf states that in *Pisum sativum* and in *Phaseolus multiflorus* and *vulgaris*, the "youngest parts" of the terminal bud do not inhibit, but that the actively elongating parts of the shoot do inhibit, and so also to some extent do the full-grown leaves and internodes.

This inhibition by full-grown parts was not detected in the experiments with *Pisum* reported above, and it is not possible to tell from the summary given by Weiskopf what may be the explanation of this discrepancy. It may be noted, however, that Weiskopf was using the cotyledonary axillaries which have less inherent tendency to grow out than those of the first leaves. It is therefore possible that the full-grown parts of the shoot may have a slight inhibiting effect which is strong enough to delay the cotyledonary axillaries to some extent, but not strong enough to delay those of the first leaves in the conditions of the experiments reported above. In any case, the results of the present paper seem to make it quite clear that in *Pisum* if the full-grown leaves and internodes inhibit at all, they can only do so very slightly in comparison with the growing leaves. The Leguminosae are not included amongst the families upon which Dostál experimented.

It is hoped to examine more closely the results of Weiskopf and of other writers in Czech when a translator can be found.

9. SUMMARY

1. In seedlings of *Pisum sativum*, the inhibiting effect exerted by the shoot upon its axillary buds comes from three or four of its developing leaves.

2. The stem apex and youngest leaf primordia, of length 1 mm. or less, do not inhibit appreciably: nor does the elongation of the stem.
3. The leaf has begun to inhibit partially at a length of 2 or 2.5 mm., and it continues to inhibit partially but strongly from length 3 to 15 mm. At about 20 mm., its inhibiting effect begins to fall off rapidly, and at the final length of about 45 mm. the leaf no longer inhibits at all, or only very slightly at the most.
4. Certain qualifications of the above statements are given in the text.
5. The situation in *Vicia Faba* and *Phaseolus multiflorus* is in a general way similar.
6. In *Pisum* the inhibiting effect exerted upon the axillary of the lowest leaf by a leaf of given size near the main apex *increases* with the age and height of the seedling.

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PROTOPLASMIC RETRACTIONS IN *BRYOPSIS PLUMOSA*

By MARGARET CHATTAWAY, M.A., B.Sc., F.L.S.

(With 1 figure in the text)

IT was suggested to the writer, by Professor Ludwig Jost, whilst working at the Marine Biological Station at Naples, that the protoplasmic retractions of *Bryopsis* might repay investigation.

Material of *B. plumosa* was easily available during the spring and early summer of 1927, and was used throughout the investigation. Experiments were made with other species of *Bryopsis*, but it was found that more accurate measurements and observations were possible with *B. plumosa* than with any other species, owing to the regular spacing and arrangement of the lateral axes.

Fresh material was divided up into pieces of convenient size, and left for 24 hours to recover from the shock of wounding. After this resting period it was in healthy and apparently normal condition; a wound plug was formed on the cut surface and the material could be used, in cool weather, for 3 or 4 days. Fresh material was however available daily, and it was seldom necessary to use older material.

Measurements were made between the main apex and the point of origin of the youngest lateral, from one lateral to the next, and of the laterals themselves. The distances were first marked on paper with the aid of a camera lucida, and then measured with a scale marked in centimetres and millimetres. The units mentioned in the tables are therefore the scale units at a magnification of a 2-inch objective and a No. 2 Leitz eyepiece.

Contractions of two kinds were noted, first shrinkage, or loss of length of the material itself, and secondly an actual retraction of the protoplasm from the wall of the siphon.

It might perhaps be expected that in a coenocytic alga such as *Bryopsis* the protoplasmic conditions would be uniform, at least throughout one small piece of material, for the laterals and main axis are in direct protoplasmic continuity throughout, but apparently such is not the case, for there was always variability in the results noted from point to point in the siphon.

WOUND REACTION

Wounding of the material caused instantaneous local retraction of the protoplasm, which spread rapidly throughout the material. Thus, upon severance of the main axis a centimetre from the apex, retraction spread in 2 or 3 minutes throughout the entire length. The rate of the spread of retraction in some of the older laterals was slower as they were more divided from the main axis. The wound reaction began immediately upon severance of the axis, and reached its maximum in about 30 minutes or less.

If one finger is placed on an undivided strand of *Bryopsis*, and the strand is then severed, the shrinkage of the strand can be felt distinctly, and may be likened to the shrinkage on cutting of a piece of stretched rubber tubing.

The average shrinkage of the siphon (for a large number of pieces) was found to be 12.5 per cent. of the original length, 5 minutes after wounding. Usually there was a slight further shrinkage for half an hour, but in one or two cases recovery began during that period, so that calculations based upon half-hour measurements may show less than the maximum retraction and shrinkage.

SHRINKAGE AND RETRACTION IN SEAWATER SOLUTIONS OF
VARIOUS CONCENTRATIONS

Except where otherwise stated the material was left in the various solutions for 1 hour.

(a) Seawater solutions of increased concentration.

Plasmolytic effects were caused by the use of various salt solutions of a concentration greater than seawater, but in order to avoid toxic effects due to the presence of unusual salts, experiments were chiefly made with more concentrated solutions of seawater.

The results were in accordance with expectation, greater concentrations causing greater shrinkage of the material.

A concentration of 10.0 per cent. above the normal strength gave 3.8 per cent. shrinkage, 12.5 per cent. above the normal gave 5.0 per cent. shrinkage, 20.0 per cent. above the normal gave 9.5 per cent. shrinkage and 50.0 per cent. above the normal gave 11.3 per cent. shrinkage, while a concentration of 60.0 per cent. above normal caused collapse in all material.

In the solutions up to 50.0 per cent. concentration above normal, with normal material, there was little or no protoplasmic retraction: occasionally the chloroplasts had withdrawn slightly from the apices

of the younger branches, but only in exceptional cases was the protoplasmic membrane withdrawn from the cell wall. In a few cases immediate collapse occurred in all concentrations, but this was always in material which had deteriorated through the excessive heat, or had been damaged in manipulation. A concentration of 60.0 per cent. above normal however always caused complete collapse of all the material used, in a few minutes.

On transference to normal seawater material from all concentrations up to 50.0 per cent. recovered almost its normal size. When material which had been left for 24 hours in a 12.5 per cent. concentration was transferred to normal seawater and examined under the microscope, immediate recovery was seen to begin.

The following table shows, first the shrinkage in 50.0 per cent. above normal concentration after 1 hour; further shrinkage after 2 hours; and then recovery of turgor in normal seawater. In the first two experiments the material was left overnight in the normal solution, and in the last two for 4 hours.

TABLE I.

Shrinkage in seawater concentrated to 50.0 per cent. above normal, and recovery in normal seawater (units of length as defined on p. 359).

No.	Portion	Length at start	After 1 hour	2 hours later	Normal seawater
I	Apex to 5th lateral	8.2	6.85	6.35	8.1
	5th to 10th lateral	7.6	7.2	6.9	7.5
II	Apex to 4th lateral	5.45	4.7	4.7	5.4
	4th to 6th lateral	5.45	4.7	4.7	5.4
	6th to 10th lateral	6.5	5.65	5.5	7.5
III	2nd lateral	3.1	2.0	1.95	2.3
	4th lateral	3.05	2.7	2.7	3.0
IV	Apex to 5th lateral	9.15	8.0	7.8	8.85
	5th to 10th lateral	6.7	5.65	5.6	6.4
	10th to 15th lateral	6.25	5.3	5.25	6.05
	7th lateral	5.8	4.7	4.5	5.25
V	Apex to 1st lateral	7.4	6.35	6.35	6.65
	1st to 5th lateral	6.65	5.7	5.7	6.0
	1st lateral	4.95	4.25	4.2	4.9
	2nd lateral	6.4	5.55	5.5	6.15
	3rd lateral	6.65	5.75	5.7	6.4
	4th lateral	6.9	5.95	5.8	6.65

(b) *Diluted seawater.*

When experiments were made with seawater of increased dilutions the expected results were a rising increase of turgor, following normal osmotic lines. The results actually obtained after 1 hour were as follows:

A dilution of 20.0 per cent. gave an increase of 3.0 per cent.;

of 25.0 per cent. an increase of 0.4 per cent.; of 33.3 per cent. a shrinkage of 4.8 per cent.; and of 50.0 per cent. a shrinkage of 0.5 per cent.; greater dilution killed and disorganised the material.

These results may seem a little variable on first sight, but upon a closer and more detailed examination they can be seen to be related to one another, and to be capable of a reasonable explanation.

In seawater of 20.0 per cent. dilution the increase in length of the material was normal, but in seawater of 25.0 per cent. dilution the measurements were very variable, not only in different pieces of material, but even in the same piece, sometimes an increase and sometimes a decrease being recorded; the preponderance being slightly in favour of the increase, thus giving the above-mentioned mean increase of 0.4 per cent. of the original length. A dilution of 33.3 per cent. gave an almost constant shrinkage, some slight increase occurring in very rare cases. This shrinkage must be due to the action on the protoplasm of a solution more dilute than its normal medium (cf. Osterhout (1)), and differs in many ways from the shrinkage caused by increased concentrations, in that the protoplasm is retracted away from the cell wall to a considerable extent, and the end product resembles the protoplasm as retracted by distilled water.

The variable results found on using a 25.0 per cent. diluted solution can be explained as depending on differences in the conditions from point to point in the siphon. It is likely that slight loss of turgor always occurs in this solution, but where the loss is very slight decreased width may be accompanied by slight gain in length, as is shown in the following diagram (Fig. 1).

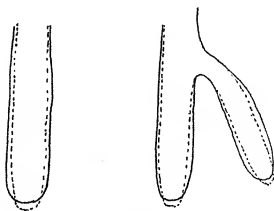


Fig. 1.

This has been confirmed by measurements and camera lucida drawings of material immersed in a 25.0 per cent. diluted solution. It occurs also in other solutions, but is soon covered by further decrease, and consequently it can only be noticed in solutions near the critical point.

Protoplasmic Retractions in *Bryopsis plumosa* 363

Material which had been in 33.3 per cent. diluted seawater for 1 hour showed a retracted appearance, but after further time in the same solution it began to assume a more normal appearance. Thus the effect of the dilute solution seems to be one of shock, from which the material gradually recovers.

Consecutive measurements show the results given in the following table; the final length of the material sometimes surpassing, sometimes falling a little short of the original one.

TABLE II.

Recovery of turgidity in seawater diluted to 33.3 per cent. below normal (units of length as defined on p. 359).

No.	Portion	Length at start	After $\frac{1}{2}$ hour	After 1 hour	After 2 hours	Over- night
I	Apex to 5th lateral	10.85	10.45	10.1	10.4	10.5
	5th to 10th lateral	9.7	9.25	9.25	9.3	9.4
II	Apex to 1st lateral	6.5	6.5	6.4	6.3	6.95
	1st to 5th lateral	5.7	5.65	5.65	5.6	5.8
	5th to 10th lateral	6.15	6.05	6.05	5.95	6.2
	10th to 15th lateral	4.8	4.7	4.7	4.65	4.8
	1st lateral	4.05	3.9	3.9	3.85	3.95
	2nd lateral	5.25	5.15	5.05	5.0	5.25
	Apex to 3rd lateral	6.2	5.9	5.85	5.9	6.15
III	3rd to 5th lateral	5.4	5.2	5.2	5.35	5.4
	5th to 10th lateral	7.95	7.7	7.7	7.8	8.0
	10th to 15th lateral	6.2	5.9	5.9	6.05	6.15
	1st lateral	2.45	2.4	2.4	2.45	2.5
	2nd lateral	3.55	3.3	3.4	3.45	3.55
	3rd lateral	4.05	3.9	3.85	3.95	4.15
	4th lateral	5.6	5.3	5.3	5.35	5.5

In these pieces of material neither the shrinkage nor the recovery were as great as in some of the pieces that were used for microscopic examination, of which the details are given below. No "excessive turgor" was noticed in these pieces (as it was in some of those microscopically examined) and the final stage seldom exceeded the original one. The material was not in good condition at the end of the experiment, though not dead.

Details of the microscopic examination are given below:

I. 15 minutes, some retraction; 30 minutes, retraction more marked; 45 minutes, no change; 1 hour, protoplasm much retracted in parts of the siphon; 2 hours, no change; 3 hours, retraction disappearing; overnight, recovery and excessive turgor.

II. 15 minutes, faint retraction in the older branches; 30 minutes, no change; 45 minutes, no change; 1 hour, retraction disappeared; 2 hours, no change; 3 hours, no change; overnight, recovery and excessive turgor.

III. 15 minutes, slight retraction; 30 minutes, distinct retraction in some branches; 45 minutes, no change; 1 hour, slight lessening of the protoplasmic retraction; 2 hours, retraction almost disappeared; 3 hours, all signs of retraction gone; overnight, quite turgid.

IV. 15 minutes, protoplasm much disorganised; 30 minutes, marked retraction; 1 hour, no change; 2 hours, lessening of the retraction, but no change in the amount of disorganisation; 3 hours, no protoplasmic retraction, still some—though less—disorganisation.

V. 15 minutes, faint protoplasmic retraction; 30 minutes, slight increase of retraction; 1 hour, no change; 2 hours, retraction disappeared; 3 hours, no change; overnight, fully turgid.

VI. 15 minutes, protoplasm much disorganised; 30 minutes, marked retraction; 1 hour, no change; 2 hours, retraction disappearing; 3 hours, retraction gone, but some slight disorganisation still left; overnight, quite turgid.

VII. 15 minutes, distinct retraction; 30 minutes, slight increase of retraction; 1 hour, no change; 2 hours, slight diminution of retraction; overnight, recovery and excessive turgor.

Similar detailed examinations were made of material subjected to treatment with 50.0 per cent. diluted seawater. Here it was found that shrinkage and retraction occurred much more quickly, but were of less duration than in the 33.3 per cent. diluted solution: increase started more quickly, and thus measurements taken at the end of an hour did not really represent the maximum retraction, but rather the stage to which the recovery of turgor had progressed. This explains why the shrinkage after 1 hour was less in the 50.0 per cent. than in the 33.3 per cent. solution, as stated above.

Details of the microscopic examination are given below:

I. 5 minutes, marked retraction; 10 minutes, no change; 15 minutes, no change; 40 minutes, no change; 45 minutes, some lessening of retraction; 2 hours, recovery almost complete; 5 hours, recovery complete, material fully turgid, but the protoplasm somewhat aggregated at the ends of the branches; transferred to normal seawater, extreme retraction.

II. 5 minutes, marked retraction; 10 minutes, some slight increase of retraction; 15 minutes, no change; 20 minutes, no change; 40 minutes, no change; 2 hours, recovery almost complete, but protoplasm aggregated at the apices of the laterals; 5 hours, fully turgid, marked aggregation; transferred to normal seawater, extreme retraction.

TABLE III.

Recovery of turgidity in seawater diluted to 50.0 per cent. below normal (units of length as defined on p. 359).

No.	Portion	Length at start	After $\frac{1}{2}$ hour	After 1 hour	After 9 hours	Over-night
I	Apex to 3rd lateral	5.45	5.35	5.4	5.5	5.75
	3rd to 7th lateral	7.5	7.35	7.45	7.9	7.95
	7th to 10th lateral	4.1	4.1	4.05	4.15	4.3
	3rd lateral	3.5	3.4	3.4	3.5	3.6
	4th lateral	5.45	5.25	5.25	5.35	5.5
	5th lateral	6.95	6.65	6.65	6.95	7.15
	6th lateral	8.3	7.6	7.6	8.1	8.2
II	Apex to 3rd lateral	6.5	6.25	6.3	6.55	6.9
	3rd to 8th lateral	8.25	7.75	7.8	8.25	8.35
	8th to 15th lateral	10.0	9.1	9.4	9.65	9.7
	2nd lateral	4.15	3.9	3.9	4.05	4.1
	3rd lateral	4.15	3.8	3.8	3.95	4.1
	4th lateral	4.95	4.75	4.75	4.85	4.95
III	Apex to 3rd lateral	5.1	4.75	4.8	4.9	4.95
	3rd to 7th lateral	No recovery in this portion				
	2nd lateral	2.3	2.0	1.95	1.95	2.1
IV	3rd lateral	3.45	3.3	3.15	3.25	3.35
	Apex to 4th lateral	6.85	6.8	6.9	6.95	7.15
	4th to 8th lateral	6.35	6.3	6.35	6.5	6.65
	8th to 14th lateral	8.25	8.25	8.3	8.45	8.6
	3rd lateral	3.35	3.3	3.4	3.4	3.4
	4th lateral	3.45	3.4	3.4	3.5	3.5
	5th lateral	6.15	6.1	6.15	6.15	6.25
	6th lateral	7.85	7.7	7.8	7.9	7.9
	7th lateral	7.6	7.3	7.4	7.45	7.45

III. 5 minutes, slight retraction; 10 minutes, no change; 20 minutes, no change; 25 minutes, some lessening of retraction; 30 minutes, retraction almost disappeared; 35 minutes, no change; 40 minutes, no change; 2 hours, protoplasm showing signs of aggregation; 5 hours, material fully turgid.

The writer does not however infer that the final conditions in this material were in any way comparable to the original ones. Material transferred to normal seawater after 5 or 6 hours in a solution diluted to 50.0 per cent. collapsed immediately.

(c) *Seawater at 100° C.*

Measurements were made of the shrinkage which occurred upon killing the material quickly. Various substances were tried, but most of them were discarded as being either too slow in action, or as causing purely physical after-effects through the withdrawal of sap from the dead cells. Eventually seawater at 100° C. was used. This caused quick killing, and no plasmolytic after-effects. The seawater was brought up to 100° C. and used immediately, so that concentration of the solution by evaporation was avoided.

The average shrinkage on killing with this solution was considerably less than the wound shrinkage, being 6.5 per cent. of the original length. Material killed in this manner showed no retraction of the protoplasm, the hot water having caused immediate coagulation.

(d) Cold distilled water.

An interesting comparison was made with material which had been killed in cold distilled water; here the toxic effects were slower in action, the final shrinkage greater, and the protoplasm much retracted, greatly disorganised and often discoloured.

The average shrinkage was 16.6 per cent. of the original length.

Details of a few experiments with distilled water are given below:

I. 5 minutes, protoplasmic retraction; 10 minutes, no change; 1 hour, no change; 3 hours, protoplasmic retraction much greater; 6 hours, death and discoloration.

II. 5 minutes, protoplasmic disorganisation, wound-plug at the cut end gave way, and the contents of the siphon flowed out.

III. 5 minutes, some retraction and shrinkage; 1 hour, marked retraction; 6 hours, disorganisation and collapse.

IV. Wound-plug gave way.

V. 15 minutes, distinct retraction; 1 hour, no change; 2 hours, no change, no protoplasmic discoloration or disorganisation, presumably not dead. On transference to normal seawater, complete collapse.

VI. 10 minutes, much retraction; 2 hours, protoplasm apparently dead, disorganised and discoloured. On transference to normal seawater, complete collapse.

These last two experiments showed that a change had taken place as death approached, such that a solution which was previously normal to the plant—as in this case seawater—caused collapse. Comparison here is interesting with the previously described experiments with seawater at 100° C., where the protoplasm was killed and coagulated, so that there was no retraction of the protoplasm on transference to normal seawater.

DISCUSSION

The first point of interest which appeared during these investigations was the extreme variability of the conditions from point to point in the siphons. Despite the fact that the material was a siphon, and that all portions were in direct protoplasmic continuity, extreme variations were observed, both in the amount of protoplasmic retraction and in the loss of turgor, as measured by the shrinkage in

length of the material. These differences were observed, not only upon wounding, but also in all experiments performed with the different solutions. They were found not only between different plants, but also between different parts of the same plant. Attempts were made to find a cause for such differences, but the above explanation of variability of conditions in the siphon seems to be the only possible one. That such differences of condition do exist has been noticed before, Osterhout (2) stating that he found not only differences between different marine plants in their response to distilled water, but also between different cells of the same plant. The writer would add to this that such variations occur also in material in which the conditions might be expected to resemble more closely those of a single cell.

Lateral branches, which had been cut off by walls, were also observed to show less retraction and shrinkage than the rest of the material. The explanation here is that the stimulus must travel from the main stem to the lateral branches, and the retraction was found to be less when the lateral branches were almost cut off by the formation of these walls at the point of junction with the main stem. Branches thus cut off eventually form new plants by vegetative propagation (West (3)) and the contraction becomes progressively less pronounced as the dividing wall is in a more advanced stage of formation; no retraction or shrinkage was observed in lateral axes which were completely cut off, and only slight contraction occurred very slowly where the dividing walls were almost but not quite completed.

There was found to be no protoplasmic retraction upon killing with seawater at 100° C., though measurement showed loss of turgor. The protoplasm was however greatly altered and coagulated, as is shown by the complete collapse of the dead siphons in any solution above the normal concentration.

The recovery of turgidity, even after long subjection to solutions of greater than normal concentration, proves that these somewhat unusual concentrations are not fatal to *B. plumosa*, and are quite possibly normal to a plant which may, in times of extreme heat, be subjected to increase of concentration through evaporation of the medium in which it lives.

After immersion in solutions of 33.3 per cent. dilution, and over, the material shows first contraction, then recovery of turgor, and in some cases increased turgor. Osterhout (1) has observed protoplasmic retractions in various water-plants when placed in distilled water, and has explained them as due to an increase in permeability

of the protoplast, which permits the solutes of the cell sap to diffuse out. The writer has observed not only protoplasmic retractions in distilled water, but also protoplasmic retractions and shrinkages in length in seawater of 33.3 per cent. dilution. Furthermore, the writer has found that after immersion in the diluted seawater recovery takes place. From this it follows that if Osterhout's explanation is correct we must further suppose that in *Bryopsis* the increased permeability of the protoplast can be subsequently reduced again, and also that in some way the concentration of solutes within the cell must then rise above that of the external medium.

SUMMARY

(1) Wounding of *Bryopsis plumosa* causes retraction of the protoplasm from the cell walls. A wound-plug is quickly formed and the material recovers its normal condition.

(2) In solutions of increased concentration shrinkage always occurs, but in solutions up to 50.0 per cent. concentration recovery follows on transference to normal seawater.

(3) In slightly diluted solutions increased turgor occurs; but in more dilute solutions "false plasmolysis" takes place. This is of the nature of a shock from which the material gradually recovers.

(4) Material killed in seawater at 100° C. shows coagulated protoplasm and collapses on transference to normal seawater.

(5) Material placed in distilled water shows marked protoplasmic retraction and is killed.

The author wishes to thank Professor Ludwig Jost for suggesting this work, Mr R. Snow for advice and encouragement, and the Board of the Faculties of Oxford University for the award of the Naples Biological Scholarship, by means of which this work was made possible.

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SEED GERMINATION AND GROWTH OF *CALLUNA VULGARIS*

By LEWIS KNUDSON

(Cornell University)

(With Plates VII-IX)

IN recent years the subject of symbiosis has received increasing attention, and one of the prominent investigations in this field is that of Rayner^(6, 7, 8) on the relation of the fungus to the heath plant *Calluna vulgaris*. These studies by Rayner were begun initially to determine the cause of the calcifuge habit of *Calluna*, but at the very outset it was apparent that attention would have to be given to the relation existing between the endophytic fungus and its host plant *Calluna vulgaris*. This involved a study of the distribution of the fungus in the host, isolation and description of the fungus and the physiological relations existing between the fungus and *Calluna*.

Some of the salient points presented by Rayner are as follows: The fungus is present not only in the roots but it grows upward through the plant and may be found in the leaves. From the stem the fungus grows into the floral organs, extending its growth from the columella and walls of the fruit to the testa of the developing seed. Only the endosperm and embryo are free of the fungus. In the roots the fungus is clearly discernible within the large cells that comprise the single layer of cortex. In other tissues the fungus is not found within the cells but in an attenuated form it is noted in the middle lamellae, and appears likewise in the intercellular spaces of the leaves. In the root cells the fungus hyphae undergo digestion, and this occurs throughout the growing season. This digestion of the fungus is similar to the digestion of the fungus hyphae in orchid roots, and with the disintegration of the fungus nuclear changes may occur in the invaded cells. For this and other reasons Rayner considered *Calluna* as a dual organism.

Rayner furthermore isolated the endophytic fungus and described it as *Phoma radialis Callunae* (8). This is given considerable significance because of the reported fixation of nitrogen in other species of *Phoma* (Ternetz⁽⁹⁾, Duggar and Davis⁽²⁾). The essential feature of Rayner's work which concerns the present investigation is the relationship

of the fungus to seed germination. According to Rayner germination of the seed of *Calluna* is entirely abnormal unless the fungus is present and infection of the seedling occurs. Without the fungus no roots are produced and the development of the stem is much restricted and abnormal. In certain experiments Rayner(6) attempted to obtain normal germination by supplying various organic compounds, but no success was noted.

In view of the unusual relationships of fungus and host reported by Rayner it seemed desirable to re-investigate the problem of the relation of the fungus to seed germination. The rather abnormal root development of the uninfected seedlings as figured by Rayner suggested that either the nutrient solution was toxic to the seedlings or that the seedlings were injured by the mercuric bichloride used in sterilising the seed. In the investigation here reported no attempt was made to isolate the organism or to study directly the relation of the fungus to germination. In view of the reported abnormal germination of seed of *Calluna* under pure culture conditions it was believed that the ground might be cleared by re-investigating this part of the problem. This paper considers therefore the germination of seed of *Calluna vulgaris* under pure culture conditions and the bearing of these results on the conclusions reached by Rayner and Christoph(1).

METHODS

The methods used in growing *Calluna* under pure culture conditions were essentially those that had been used previously for the germination of orchid seed. The nutrient solution used was made after the formula of Rayner's solution A, as used by her. To this was added 1.5 per cent. standardised agar. This solution has the following composition:

Potassium nitrate KNO_3	1.0 gm.
Magnesium sulphate MgSO_4	0.4 "
Calcium sulphate CaSO_4	0.5 "
Calcium monophosphate $(\text{CaH}_2\text{P}_2\text{O}_6)$	0.5 "
Sodium chloride NaCl	0.5 "
Ferric chloride FeCl_3	trace
Water...	2000 c.c.

No tests were made of total acidity, as in the experimental work the hydrogen-ion concentration was adjusted to certain definite values by the use of decinormal HCl and NaOH. In certain experiments 2 per cent. glucose was added to the nutrient solution.

For sterilising the seeds use was made of calcium hypochlorite as described by Wilson(10), a method which has given very satis-

factory results in our laboratory with a large number of different kinds of seeds. The solution used is made by adding 10 grm. of calcium hypochlorite to 140 c.c. distilled water. The mixture is shaken and then filtered. The filtrate is of course used. Generally the seeds of *Calluna* were left in the filtrate for 30 minutes and then transferred directly from the solution to the culture tube without previous rinsing. The seeds were transferred to the tubes by means of a looped platinum wire. Only one seed at a time was transferred except for the first experiment.

The seeds were obtained from six plants growing in the nursery of the Department of Floriculture of the College of Agriculture at Cornell. These plants had been growing in the nursery for some years and had a spread of from 12 to 18 inches. Examination of the roots in the month of July revealed typical infection of the young roots. The fruit was stripped from the stems and placed in bottles during the month of November.

Experiment 1. In the first experiment Rayner's solution was used with 1.5 per cent. agar, and the hydrogen-ion concentrations of the various cultures were varied, as is indicated in Table I. In this experiment no effort was made to isolate the seed from the floral tissues. The dried fruiting stem was ground by rubbing with the hands and the finer material collected by sifting. A mass of the finer material which contained the seed as well as fragments of floral tissue was treated with the solution of calcium hypochlorite. Not only were seeds introduced into the tubes, but fragments of tissue as well. The result was that every tube showed a contamination. Owing to the lack of sugar the fungus growth was slight. Examination of the contaminating organism revealed that it was a species of *Alternaria*, and in no case was found the characteristic *Calluna* organism described by Rayner. The growth of the *Alternaria* was sufficient however to change the pH value of the culture medium, and growth of the seed was somewhat erratic. No conclusion is possible in this experiment as regards the relation of the hydrogen-ion concentration to germination and growth of the embryo. Not all of the data are presented, but the data on the best seedlings in each culture are given in Table I.

The roots of many of the plants were examined microscopically, and in no case was any fungus observed in the transparent cortical cells. All of the plants described in Table I were fixed in chrom-acetic fixative, imbedded in paraffin, and permanent slides were prepared. The procedure followed was the same as used for orchid

TABLE I

Growth of *Calluna* seedlings. Duration, December 4th, 1926, to March 16th, 1927. Cultures contaminated with *Alternaria* sp.

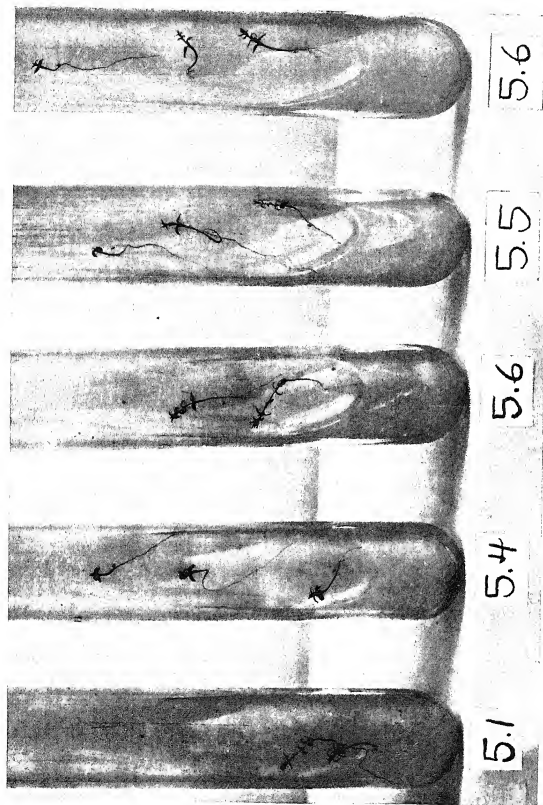
Initial pH	Final pH	Length of tops (mm.)	Length of roots (mm.)
4.5	5.8	8.0	5.2
4.7	6.0	9.8	8.6
4.9	6.1	12.4	10.4
4.9	5.8	8.7	7.7
5.1	5.9	12.6	9.4
5.3	5.5	9.4	32.0
5.3	5.7	12.2	14.1
5.75	5.9	10.1	12.7
5.75	5.5	9.7	7.7
5.91	5.1	9.7	14.6
6.8	6.2	11.0	9.5
6.8	5.5	8.2	15.0
7.5	5.3	12.6	3.5

seedlings (Knudson(5)). The sections were stained in Haidenhain's iron-alum-haematoxylin. Careful microscopic examination was made of these slides, but in no case was any root infection observed. Adhering to the surface of some of the root sections were hyphae and spores of *Alternaria*, and these were well stained.

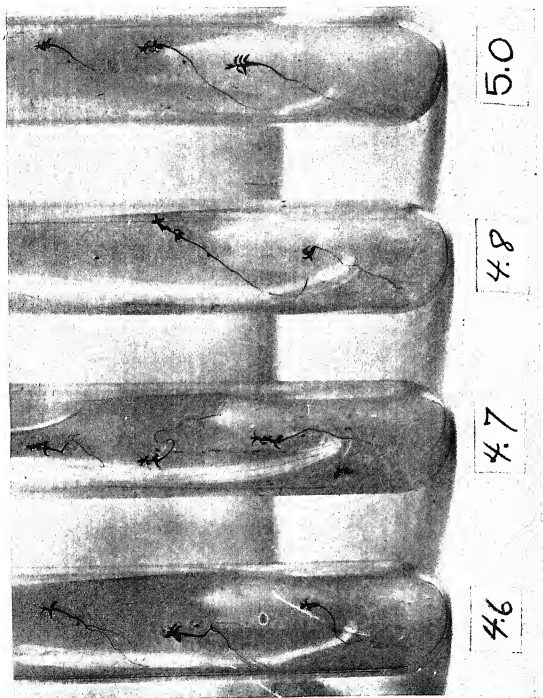
The data of this experiment are significant because of the marked development of some of the seedlings without fungus infection. Furthermore, the roots were of a characteristic healthy appearance, being glistening white in colour.

Experiment 2. Because of the contaminations in the preceding experiment it was decided to remove all foreign matter from the seed. By careful hand picking, sound seeds were selected for the second experiment. The seeds were sterilised as before, and in each tube were placed three seeds. Rayner's solution with 1.5 per cent. agar was again used with the hydrogen-ion concentrations adjusted by the addition of definite amounts of sodium hydroxide and hydrochloric acid. The experiment was begun on April 22nd, 1927, and completed on July 9th, 1927. The results and certain details of the experiments are given in Table II, and the various cultures at the conclusion of the experiment are shown in Plates VII and VIII.

In place of giving average figures the data on each seedling that developed are presented. In tube cultures it often happens that the seedling which becomes first established succeeds in maintaining its supremacy. This is probably due to the limited availability of CO₂ in the tubes, so that the plant with the larger leaf area obtains a greater amount of CO₂ than the plant with the smaller leaf area.



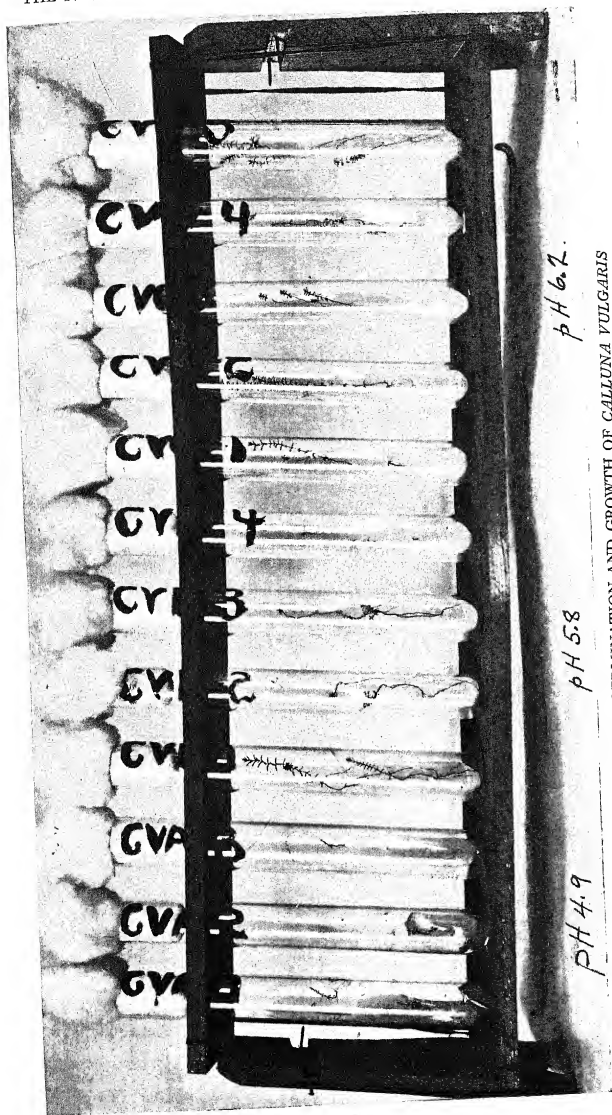
Face p. 372



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KNUDSON—SEED GERMINATION AND GROWTH OF *CALLUNA VULGARIS*





KNUDSON—SEED GERMINATION AND GROWTH OF *CALLUNA VULGARIS*



TABLE II
Germination and growth of *Calluna vulgaris*

Tube symbol	Desired pH	pH after sterilisation	Final pH July 9th	Length of tops (mm.)			Length of roots (mm.)		
				$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$
L	4.5	4.7	4.6	10.0	11.0	13.0	10.0	23.0	14.5
M	4.7	4.6	4.7	19.0	3.5	—	17.2	18.3	—
N	4.9	4.7	4.8	8.0	12.0	12.0	3.0	17.0	37.0
O	5.1	5.1	5.0	16.0	12.0	—	23.0	27.0	—
P	5.4	5.2	5.1	7.0	9.5	11.5	14.0	32.0	10.0
Q	5.7	5.5	5.4	14.0	21.0	—	15.0	20.0	—
R	6.0	5.75	5.6	6.8	13.0	8.0	21.0	29.0	13.0
S	6.3	5.8	5.5	7.3	8.8	16.3	19.0	5.5	5.5
T	6.6	5.8	5.6						

From the data presented it is apparent that no one hydrogen-ion concentration was more favourable for growth, except that in the cultures *R*, *S* and *T* the plants were not as green in colour as with the higher hydrogen-ion concentrations. This is due, as has been shown by Hopkins and Wann (3), to a lack of available iron.

It will be noted that in some cases not only were the roots of considerable length but none of the plants showed the characteristic root knobs described by Rayner (6) for plants not infected.

To determine whether or not any of the roots were infected, roots from one or more plants in each tube were examined microscopically in the living condition. The remaining plants were fixed, sectioned and stained for examination. Hundreds of slides were made, but no infected cells were found. Furthermore, no fungus was found growing from the seed testa, and the agar surfaces were free of any microbial growth. The conclusion is inevitable from this and the preceding experiment that the fungus is not essential for normal germination of the seed.

OTHER EXPERIMENTS

Another experiment similar to experiment No. 2 was made, with the exception that only three different hydrogen-ion concentrations were used, pH 4.9, pH 5.8 and pH 6.2. Four tube cultures were made in each series. The experiment was begun on May 14th, 1928, and concluded on July 13th, 1928. The results as regards growth are shown in Plate IX. Again there was no evidence of any fungus growth. The roots of these plants were used entirely for prepared sections. Careful microscopic examination did not reveal any infected cells.

Since the results obtained in these experiments were so at variance with those reported by Rayner it seemed desirable to examine the plants from which the seed was obtained to see if mycorrhiza were

present. Roots were obtained from various plants growing in the nursery, and these with their young transparent roots were examined microscopically. There was no difficulty in detecting the fungus in most of the roots that were examined.

In connection with the third experiment it will be noted from Plate IX that the cultures of pH 4.85 were less vigorous than others. This may have been due to toxicity caused by a slight excess of iron. With the higher pH values some of the iron would be precipitated.

DISCUSSION

The results here reported are in complete opposition to the results reported by Rayner. The seed germinated readily and normally when supplied with Rayner's solution and in the absence of the endophytic fungus. Examination of the figures will reveal that the seedlings produced splendid roots. They were glistening white in appearance, of good length and branched. No evidence of a toxic condition was noted. In Rayner's experiments no roots were produced in the absence of the fungus, but slight protuberances were noted in place of roots. With the fungus supplied Rayner (8) obtained root development, but the roots produced were still somewhat abnormal in appearance in certain of her experiments.

The facts as reported by Rayner are of course accepted. The question that naturally follows is, what is the explanation of Rayner's results? Two explanations are possible, both involving toxicity as a cause of lack of root development.

The toxicity may be due to an excess of iron. Rayner's solution A as made up by us has a pH value of 4.85. It might go lower or be slightly higher, depending on the quantity of iron added and the quality of phosphate used. With this reaction iron is maintained in an available form, and if the amount of ferric chloride approaches 40 mg. per litre toxicity may result. This may possibly be a factor in Rayner's experiments with solution A, but obviously it cannot hold for those experiments in which the seedlings were grown on filter paper supplied with distilled water. In these experiments the fungus was likewise effective in inducing root development, and without the fungus roots were not formed. Unfortunately there is little evidence on this aspect of the work.

The more probable explanation for the abnormal behaviour of uninfected seedlings is that the seeds were injured by the mercuric bichloride used in sterilisation. For this purpose they were first

treated with 1 per cent. mercuric bichloride and then rinsed in distilled water.

As regards this method of sterilising the seed Rayner writes as follows: "If due precautions are observed, seeds can be sterilised without injury to the embryo by washing in 1 per cent. corrosive sublimate solution. Complete sterilisation is not easy to effect, and the margin of safety is a narrow one, owing no doubt to the delicacy of the testa and the fact that infection of the cells of the seed coat is more extensive and deep seated than is the case with air-infected seed. If seeds were kept a few seconds too long in the sterilising solution the embryo was killed outright, germination was delayed or the seedlings which germinated showed complete chlorosis" (*Ann. Bot.* 29, p. 105).

In referring to the growth of uninfected seedlings Rayner (*Ann. Bot.* 29, p. 106) states as follows: "The seedlings usually formed few leaves, chlorotic or reddish in colour, but did not develop roots, although they remained in a turgid condition and apparently alive for five to six months."

It is quite apparent that the method of sterilising the seed may lead to trouble, since an exposure of a few seconds too long may result in death. The assumption appears warranted, particularly in view of my own experiments in which calcium hypochlorite was used for sterilising the seed, that the reason for the failure of root development is injury caused by mercuric chloride. This may be due to injury during the process of sterilising the seed, and possibly to a carry over on the seed coat of some of the mercuric chloride.

This leaves unexplained the effectiveness of the fungus in inducing root formation. It is conceivable that a change in reaction about the seed coat may result in a release of mercury which becomes dissipated by diffusion through the culture medium. There is likewise the possibility that the mercury may form double salts with substances produced by the fungus and the toxicity of the mercury thereby decreased.

Whatever may be the real explanation for the effectiveness of the fungus in inducing germination under the conditions described by Rayner, the fact remains that seed of *Calluna vulgaris* will germinate without any fungus. Such seedlings are normal in every respect. The contention of Rayner that obligate symbiosis is a requisite for seed germination is therefore not tenable.

My results tend to confirm the observations reported by Christoph⁽¹⁾ that seedlings may develop without the fungus.

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SEEDLING DEVELOPMENT IN *CALLUNA* *VULGARIS*

By M. C. RAYNER

APPEARING simultaneously with this review is a paper by Knudson entitled "Seed Germination and Growth of *Calluna vulgaris*" (*ante*, p. 369).

The conclusions reached are so markedly at variance with those first placed on record by myself in 1915 that the observations and experiments on which they are based challenge immediate and careful examination.

The point at issue is a simple one. Can seedlings of *Calluna vulgaris* develop normally when produced from *adequately* sterilised seeds, under the completely controlled conditions described by myself in 1915 and by Knudson in the paper under discussion?

The evidence that they do not do so, but that root development is induced and growth resumed by bringing such seedlings into contact with a pure culture of the endophytic fungus under appropriate conditions, has been published in full detail and need not be recapitulated (Rayner, 1915, 1925, 1927).

Extension of the conclusions based on this evidence to plants growing under natural conditions is fully justified, not only by the presence of the endophyte within the fruit chamber and its close association with the seeds, but also by the early and invariable association of characteristic mycelial infection with seedlings raised from untreated seeds, and, even more strikingly, by the appearance of similar phenomena in viviparous seedlings produced from seeds that have germinated precociously within the fruits. The latter was noted by Ternetz as a not uncommon occurrence in *Andromeda polifolia* and has been observed by me—in a single instance in each case—in *Calluna vulgaris* and *Pernettya mucronata*. Although attention is not directed to this matter by Knudson, there can be no doubt whatever of the fact of early and specific infection of *Calluna* seedlings raised from seeds removed from intact fruits; the question in dispute, therefore, is the physiological significance of such infection.

The possibility that germination proceeds differently in different locations: that *Calluna* plants growing in Bavaria (*vide* Christoph, 1921) behave differently in this respect from those growing in the British Isles but similarly to others growing in the United States, is a possible but not, in my view, a tenable hypothesis.

As a preliminary step, exception may be taken to certain minor inaccuracies of statement. For example, the following cannot be justified: "According to Rayner *germination of the seed of Calluna is entirely abnormal unless the fungus is present and infection of the seedling occurs.*"¹

On the contrary, it has never been suggested that germination of adequately sterilised seeds is other than perfectly normal, as are also the early stages of seedling development. Germination takes place at the 18th to 21st day as with untreated seeds. The minute embryo absorbs food material from the endosperm, and increases rapidly in size to form a normal seedling consisting of a short hypocotyl bearing two cotyledons and terminated at either end respectively by plumular bud and short blunt radicle. At this stage there is a pause in development, easily discernible in seedlings germinating under natural conditions; in those produced from properly sterilised seeds, root development is inhibited and this is followed by arrest of growth and the inevitable symptoms of malnutrition.

Again, in relation to the possibility of effecting asymbiotic seedling development by artificial means, it is misleading thus to represent my considered views by citing those published in 1915—"Rayner attempted to obtain normal germination by supplying various organic compounds, but no success was noted." For various reasons the line of work indicated was not pursued to a conclusion and the results of subsequent experiments remain unpublished. The effects on seedling development produced by planting, e.g. in fungus cultures killed by heating, and in others containing various organic substances, were sufficiently promising, however, to leave little room for doubt that the raising of seedlings by such methods could easily be accomplished.

This opinion is sufficiently indicated in the following extracts:

Attention has already been drawn by me to the possibility of replacing the stimulus to development normally provided by the fungus by stimuli of a chemical nature, e.g. by the addition of organic substances to the rooting medium in which seedlings are

¹ *Italics are mine.* M. C. R.

growing. It is even conceivable that an appropriate organic substance might be present in sterilised peat and so provide an explanation of the discordant experimental results recorded by Christoph (Rayner, 1922, p. 65).

And, again,

The possibility or otherwise of replacing the stimulus to development ordinarily supplied by infection by the addition of a suitable organic substance to the rooting medium has not yet been fully explored in Ericaceae. In view of the asymbiotic germination of orchid seeds when supplied with appropriate organic material, it seems reasonable to infer that similar methods might be used successfully in Ericaceae, and that seedlings thus raised free from infection might grow satisfactorily without mycorrhiza (Rayner, 1927, p. 100; see also Rayner, 1925, pp. 287-8).

The present seems to be a suitable occasion to reiterate the opinion that the raising of seedlings of *Calluna* by asymbiotic methods will be found to depend upon artificially providing the appropriate conditions. At the same time, it appears to me most improbable that either for *Calluna* or for orchids, such appropriate conditions ever exist in nature apart from those produced by proximity to the specific endophytes.

Passing to certain criticisms offered in the paper under review Knudson accepts the experimental results placed on record by me—"The facts as reported by Rayner are of course accepted"—and seeks to reconcile them with his own by two "explanations"—or more correctly *hypotheses*—"both involving toxicity as a cause of lack of root development."

The first assumes toxicity due to excess of iron in the salt solution used for agar cultures.

The solution referred to (Solution A, Rayner, 1915, p. 106) has been used freely for water cultures of *Calluna*, and has been also similarly used by Coville (1910) and by myself for *Vaccinium* without providing the slightest evidence of toxicity. The amount of ferric chloride present, recorded as "trace," never exceeded three or four drops of 0.1 per cent. solution per litre. The addition of ferric chloride to culture media at the rate of "40 milligrams per litre" is not a procedure likely to be contemplated by any responsible biologist!

The "explanation" based on this assumed toxicity does not merit serious criticism. Is it really suggested that two sets of pure culture seedlings of *Calluna* transferred simultaneously from the same seed dish to tubes of nutrient agar filled from the same flask react

differently to the rooting medium: that those inoculated from a pure culture of the endophyte at planting immediately form roots and grow normally while those lacking the fungus are all poisoned by excess of iron in the rooting medium?

This ingenuous "explanation," we are told, "obviously cannot hold for those experiments in which the seedlings were grown on filter paper supplied with distilled water." "Unfortunately," it is added, "there is little evidence on this aspect of the work." The experiments referred to are simple but crucial. Pure culture seedlings were transferred from seed dishes, some to tubes of nutrient agar, some to cones of filter paper dipping into water. In both cases those in contact with mycelium of the endophyte rooted and grew while the uninoculated controls failed to do so. Examples from both types of culture were carefully photographed and have been reproduced to illustrate an account of the work (Rayner, 1915). What further evidence is desired?

"But," it is continued, "the more probable explanation for the abnormal behaviour of infected seedlings is that the seeds were injured by the mercuric bichloride used for sterilisation....The assumption appears warranted...that the cause of the failure of root development is injury caused by mercuric chloride." The assumption is a large one, for we must account for the remarkable fact that only those seedlings *not inoculated at planting* were subjected to "injury during the process of sterilising the seeds, and possibly to a carry over on the seed coat of some of the mercuric chloride." Knudson anticipates this objection by suggesting that the addition of the fungus at planting may effect a change in reaction about the seed coat resulting "in a release of mercury which becomes dissipated by diffusion through the culture medium. There is likewise the possibility that the mercury may form double salts with substances produced by the fungus and the toxicity of the mercury thereby decreased"!

This ingenious suggestion—if it can be verified experimentally—offers an interesting starting-point for enquiry into the capacity of the root fungus to protect the roots from the effects of soil toxicity in general.

As this matter of seed sterilisation will be discussed in a later paragraph in relation to Knudson's experiments, it need only be noted here that the method used for sterilising seeds of *Calluna* was adopted after an exhaustive series of tests with other sterilising agents including mercuric chloride in weaker concentrations. The risks involved in using a 1 per cent. solution of the latter are obvious

and can be justified only in the case of seeds extremely difficult to sterilise effectively. As the technique became more familiar it was learned that these risks were almost entirely related to inadequate rinsing of the sterilised seeds. In seeds such as those of *Calluna* in which the embryo is embedded in a mass of oily endosperm, I believe there is little or no risk of injury from the use of toxic aqueous solutions however concentrated. There is, however, considerable danger from carrying over toxic substance in the seed coat, especially when the outer walls, as in *Calluna* and its allies, possess an external layer of pectose character. This risk can only be eliminated by repeated rinsings in changes of sterilised water. It is not easy to devise a satisfactory technique for the removal of all traces of the sterilising agent in the case of small buoyant seeds without subjecting them to undue risk of casual contamination before sowing.

Turning now to the new experiments on the results of which are based the criticisms contained in the paper under consideration, Knudson concludes as follows: "Whatever may be the real explanation for the effectiveness of the fungus in inducing germination under the conditions described by Rayner, the fact remains that the seed of *Calluna vulgaris* will germinate without any fungus. Such seedlings are normal in every respect. The contention of Rayner that obligate symbiosis is a requisite for seed germination is therefore not tenable."

If we ignore the inaccuracy of statement already referred to, and substitute *seedling development* for "*germination*," the meaning of this paragraph is clear.

Holding, as I do, that it is quite impossible to reconcile the results obtained by myself with those now recorded by Knudson it becomes necessary to subject his experiments to close and critical scrutiny.

1. *Sterilising methods*

The calcium hypochlorite method as used by Knudson has been tested and found inadequate and unsatisfactory for *Calluna* and other ericaceous seeds. Some, e.g. those of *Vaccinium*, from the nature of the case, cannot be freed from specific contamination by this or any other sterilising method (Rayner, 1929). The use of 1 per cent. mercuric bichloride was adopted after long experimenting with various sterilising agents including calcium hypochlorite and similar chlorine preparations.

In this matter Knudson has to reckon not only with my observations but also with those of Ternetz (1907).

To Ternetz it was of vital importance to obtain seedlings free

from contamination, a feat never accomplished however drastic the sterilising methods used. "Wenn die *Calluna*-Pflänzchen...eine Höhe von 2.5-3 cm. erreicht hatten so konnte nicht ein einziges, wirklich pilzfreies Exemplar ausfindig gemacht werden...."

Owing to their buoyancy, the character of the testa, and the intimate nature of infection, seeds of *Calluna* are extremely difficult to sterilise effectively. Unfortunately, Knudson gives no details of the methods used by him to overcome these difficulties, but in view of the published results of Exp. 1, it is clear that the technique adopted was altogether inadequate.

2. Experiment 1.

It is difficult to understand why the results of this experiment and the accompanying table were published in detail in a paper expressly dealing with "the germination of seed of *Calluna vulgaris* under pure culture conditions." They serve but to demonstrate that (a) the technique used for cleaning and sterilising seed was defective; (b) all cultures were contaminated by *Alternaria*; (c) owing to the lack of sugar the fungus growth was slight.

A primary requirement of critical work with pure cultures is to provide evidence of their purity. In the case of my original cultures, sterilised seeds were sown upon a medium rich in sugar and nitrogenous material. "As an additional test of sterility seedlings were subsequently transferred singly from seed dishes free from any trace of microbial growth to tubes of glucose broth and other media, and kept under observation for three weeks." These tests of purity were checked independently by a competent bacteriologist. Only after such rigid testing were similar seedlings transferred to tubes of nutrient agar containing the requisite inorganic salts. Satisfactory evidence of freedom from contamination cannot be provided by sowing seeds upon a substrate too poor to encourage the growth of any micro-organisms present.

Remains of the flowers and fruits of *Calluna* are heavily infected with mycelium of the endophyte and also with that of other fungi, and the seed to be sterilised must be carefully freed from all such debris. As has been pointed out in a recent paper, species of *Cladosporium* and *Alternaria* are constant impurities in seed cultures of *Calluna*. If present, mycelium of either quickly overgrows that of the endophyte.

In particular, contamination by mycelium of *Alternaria* is difficult to avoid when removing seeds from intact fruits in order to

obtain pure cultures of the endophyte. In my cultures, mycelium of this genus has on more than one occasion overgrown and replaced that of the endophyte after sub-culturing what appeared to be a pure colony of the latter. In the same paper will be found a full account of the method used to isolate the endophyte from intact fruits and the difficulties encountered in promoting independent growth of mycelium associated with seeds (Rayner, 1929 b, p. 63).

To one familiar with imperfectly sterilised seed cultures of *Calluna* the presence of *Alternaria* offers convincing evidence that the sterilising methods used were inadequate to destroy the mycelium of the endophyte present on the seed coats, while the poverty of the substrate used for the seed bed ensured that only the stronger growing mycelium would be in evidence.

This view is duly confirmed by the observation that all the experimental seedlings in these cultures rooted normally.

Moreover, to those familiar with the facts, failure to observe root infection is not difficult to understand.

In imperfectly sterilised cultures in an aseptic rooting medium typical mycorrhiza is not formed; mycelium is casual in distribution, often very sparsely developed, and may be extremely difficult to put in evidence in the earlier stages of growth. Frequently I have been tempted to believe it absent; never have I failed to find proof of its presence with the help of a suitable technique and patient investigation. In my experience the negative evidence yielded by examination of microtome sections of the roots is quite valueless. The early stages of infection in cultures such as those described show no hyphal complexes within the root cells, while the fineness of the mycelium, its casual distribution and liability to be washed away during manipulation render such preparations useless. The examination of typical mycorrhiza is another matter, although this can, in *Calluna*, also be more satisfactorily achieved by other methods (Rayner, 1925, p. 284).

Not only are we informed that "every tube showed a contamination" but it is actually stated in reference to permanent preparations of roots of these seedlings: "Adhering to the surface of some of the root sections were hyphae and spores of *Alternaria*," although it is insisted in the sentence preceding this that "careful microscopic examination was made of these slides but in no case was any root infection observed."

It is to be hoped that Knudson will supplement the present paper by one explaining exactly what he understands by "root infection"

of *Calluna* seedlings, how he differentiates casual non-sporing hyphae of *Alternaria* sp. from those of *Phoma radialis Callunae*, and incidentally, where the *Alternaria* came from if the methods of seed sterilisation and culture are to be accepted as adequate to provide pure cultures.

The two phenomena—infection of the seedling at germination and the formation of mycorrhiza—are distinct; the former is invariable, the latter conditioned by the nature of the rooting medium. The evidence for this view has been very fully stated and insisted upon in recent papers (Rayner, 1925, 1927, pp. 99, 211).

Presumably, by "root infection" Knudson understands the formation of the characteristic hyphal complexes found in typical *Calluna* mycorrhiza. In my experience, these are never formed in a sterile agar rooting medium of the kind used and only very imperfectly developed by either seedlings or cuttings in the more natural conditions provided by aseptic sand cultures (see Rayner, 1925, pp. 282-5; 1927, p. 211). The account provided of this one experiment renders further criticism needless, since it shows a complete lack of appreciation of the nature of the problems presented by the distribution of mycelium of the endophyte throughout the tissues of ericaceous species such as *Calluna* and *Vaccinium*.

Were the matter so simple as is suggested the character of seed infection in the latter genus might have been established with certainty in ten minutes instead of claiming much time during the same number of years! (Rayner, 1929 a).

In respect to the other experiments described it need only be noted that in no single case is there offered any proof or even evidence of adequate seed sterilisation beyond the statement that in roots of the resulting seedlings "no infected cells were found."

Christoph's observations on *Calluna* have already been subjected to full and independent review (Rayner, 1922). Knudson's conclusions tend to confirm those reported by Christoph because he has employed a similar technique and does not appreciate the true character of seedling infection as distinct from mycorrhiza formation. The presence and quite casual distribution of mycelium in young seedlings, especially those raised from imperfectly sterilised seeds, or the demonstration of "suppressed mycorrhiza" in older plants or rooted cuttings growing in aseptic rooting media, is difficult and demands a specially careful technique.

The fact that the presence of mycelium was overlooked in seedlings of *Vaccinium* growing in sterilised peat by so careful and competent an observer as Stahl sufficiently proclaims it (Rayner, 1929 a).

In conclusion I would ask of Knudson the following questions:

1. Has he observed the further development of seedlings raised as he describes when transferred to peat sterilised by repeated steaming followed by leaching with sterilised rain water?

2. Has he studied the condition of the roots of seedlings raised from *unsterilised* seeds in aseptic sand cultures or on peat sterilised as described above?

3. Has he observed the germination of *Calluna* seeds removed from intact fruits and sown upon filter paper or glass wool moistened with rain water and maintained under aseptic conditions?

One fact recorded in Knudson's paper is of potential interest. The material used was obtained from plants growing in the nursery of the Department of Floriculture of the College of Agriculture at Cornell, and they are reported as showing typical mycorrhiza.

We have at present no evidence bearing on the possible distribution of *Phoma radialis* as an independent soil fungus and it would be of interest to obtain such; moreover, the forms associated with *Calluna* and certain other ericaceous species are highly specialised strains not interchangeable in respect to their particular host plants.

In view of the distribution of *Calluna vulgaris* in North America, it would be of interest to have further information as to the origin of these plants at Cornell.

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NOTE ON THE USE OF THE TERM "SUCTION PRESSURE"

In the footnote on p. 39 of the admirable English edition of Professor Maximov's book *The Plant in Relation to Water* there occur some statements which call for comment and correction.

In dealing with the suction pressure of the cell it is stated: (1) that I define suction pressure (*Permeability*, 1924, p. 98) as the resultant of all the pressures concerned in the passage of water into and out of the cell, including the pressure of the external solution; (2) that this use of the term is open to objection for it implies that the suction pressure is zero when the cell is in a state of equilibrium; and (3) that it is better to limit the term "suction pressure" of a cell to the sense $S = P - T$ (suction pressure = osmotic pressure of the cell less turgor pressure), that is, the pressure sending water into the cell when this is immersed in water. I will deal with these various statements in order.

(1) On reference to the passage in *Permeability* mentioned in the footnote in Professor Maximov's book, it will be seen at once that I took some care to distinguish between the "net suction pressure," the pressure whatever the external liquid, and the "full suction pressure" when the external liquid is water. Further, I stated: "When the term 'suction pressure' alone is used... it indicates the full suction pressure." The term I proposed for the resultant of all the pressures concerned is therefore "net suction pressure," not simply "suction pressure" as stated in the footnote in Professor Maximov's book. The term "suction pressure" I proposed for another equally definite quantity. It is very necessary to have terms to distinguish between these two quantities.

(2) The actual pressure sending water into the cell (the "net suction pressure" according to my terminology) is zero when the cell is in a state of equilibrium. Even if the term "suction pressure" had been used for this (which was not the case), I can see no objection to a definition because it implies the pressure is zero when it actually is.

(3) As already mentioned, I defined the term "suction pressure" as the full suction pressure, the pressure sending water into the cell when this is immersed in water. The proposal in the third of the statements cited above therefore, so far from varying my definition of suction pressure, accepts it exactly.

Professor Maximov's book itself and the editorial footnotes supplied by the late Professor Yapp are on the whole so excellent that the book is likely to remain the standard work in English on the water relations of the plant for many years to come. For this reason it appeared to me all the more necessary to comment now on the mis-statements concerning my definition of suction pressure.

WALTER STILES.

THE UNIVERSITY, READING.

13 July, 1929.

REVIEW

Totius Orbis Flora Photographica Arte Depicta. Vol. II. Floral Province of the European "Mittelgebirge." Brno, 1929.

The progress of plant ecology is shown by the publication of numerous books and papers on the subject at the present time. Of these the work bearing the above title promises to be one of the most important. Its aim is no less than to describe the vegetation of the whole earth as shown in its typical plant associations, these latter being illustrated throughout by original photographs. In each volume a certain floristic area will be dealt with on the basis of Engler's classification, preceded by an introductory phytogeographical description. The international character of the work is shown by the fact that it is being brought out in three separate editions: German, French and English. Two to three volumes are promised annually. The editor is Dr Hugo Iltis of Brno (Brünn).

Vol. I has already appeared. It deals with Trinidad and the West Indies, and is by Professor Domin of Prague.

The present Vol. II, by Dr Iltis and Mr Schulz, is concerned with the floral province of the European "Mittelgebirge," and more particularly with Moravia and Bohemia. An introductory sketch deals with the phytogeography of the districts (chiefly Moravia), including a reference to the climate and geology, as well as to the origin and distribution of the flora. Then follows a succinct classification of the plant formations or associations, consisting of twenty-five different types, such as pine forest, heath, siltings, water plants, ruderal formations, etc. The main part of the volume is occupied by an instructive and interesting account of each of these plant communities. The main feature of the volume is the portrayal, by means of 100 original photographs of high merit, of groups of plants or, it may be, of a single plant, belonging to each community, as they appear growing in their natural habitats. Above each photograph the name of the association is given in three languages, and the locality in which it was taken; at the foot are the names of the plants composing the community, with the dominant ones in heavy type. This graphic representation of the vegetation is the unique feature of the work and will render it invaluable to students of ecology.

The volume concludes with a bibliography, an index of the photographs, a list of abbreviations in the explanation of the plates, an index of plant names, and a very clear, coloured map of the western portion of the Czecho-Slovakian Republic, explaining the phytogeographic conditions and the most important localities mentioned in the volume.

W. C. W.

SUB-COMMISSION FOR PEAT SOILS

We have been asked to publish the following communication:

A MEETING of the Sub-Commission for Peat Soils will take place in connection with the Second International Congress of Soil Science, to be held June 1st to 10th, 1930, at Moscow and Leningrad, Union of Socialist Soviet Republics.

The sessions will be devoted to the reading and discussion of papers dealing with the stratigraphy, profile analyses and cultural operations of peatlands.

Scientists and members who are interested or may wish to attend the meetings are invited to send a brief summary of their papers to Prof. Dr A. A. JARILOV, President of the Organising Committee, Gosplan, Karuninskaja 1, Moscow, U.S.S.R.

A. P. DACHNOWSKI-STOKES.

President, Sub-Commission for Peat Soils.

WASHINGTON, D.C. *October 1929.*